Synthesis and Biological Evaluation of Macrosphelide Cores

Kentaro Ishihara,[a] Takanori Kawaguchi,[a] Yuji Matsuya,[a] Hiroaki Sakurai,[b] Ikuo Saiki,[b] and Hideo Nemoto*[a]

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The simplified 16-membered core structures of macrosphelides were synthesized in high overall yields starting from methyl (+)- or (−)-3-hydroxybutyrate as the sole chiral source. The antiproliferative activities of these macrosphelide cores against murine colon 26-L5 adenocarcinoma cells were examined, and the results suggest that the oxygen functional groups found in the natural products are essential for their bioactivities.

Introduction

Recently reported natural macrolides, the macrosphelides (Figure 1),[1] exhibit potent inhibitory activity against adhesion of human leukemia HL-60 cells to human-umbilical-vein endothelial cells (HUVECs). Adhesion of tumor cells to the vessel wall endothelia of distant organs is a critical step in tumor metastasis, and consequently, macrosphelides have been recognized as a promising lead compound for new antimetastatic agents. In fact, macrosphelide B has been reported to suppress the metastasis of B16-BL6 mouse melanoma cells to the lung in vivo through inhibition of cell adhesion mediated by sialyl Lewis^x.[2] It was suggested that the antimetastatic activity did not arise from direct cytotoxicity since inhibition of tumor cell growth was not observed, and the efficiency of the combined therapy of macrosphelide B with cisplatin, a potent antitumor agent, on lung metastasis was also reported.[2] Thus, new anticancer agents that possess both cell adhesion inhibitory and antiproliferative activities are desirable.

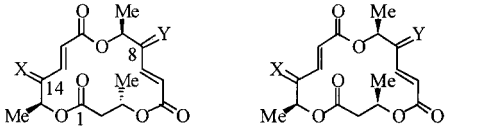


Figure 1. Representative examples of natural macrosphelides

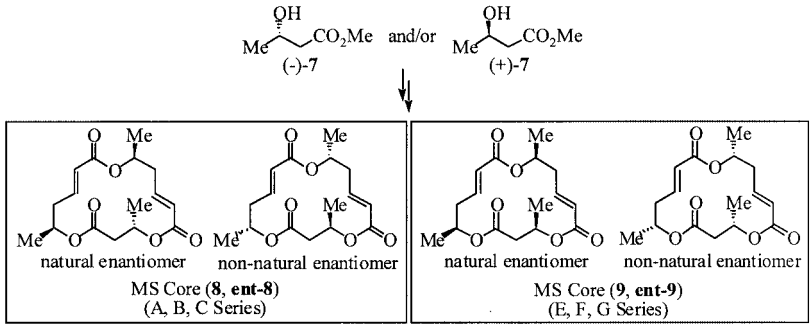
Macrosphelides, which are characterized by a 16-membered trilactone linkage, are isolated from *Macrosphaeropsis* sp. FO-5050 and *Periconia byssoides*.[1] Synthetic studies of these natural products have been performed by several groups,[3] and include the combinatorial synthesis of a 122-membered macrosphelide library.[4] However, the biological profile of the artificial macrosphelide derivatives has not been reported yet. In our ongoing research, we briefly described the synthesis and the derivatization of simple 16-membered macrosphelide core structures (abbreviated as MS Core), in which the oxygen functions found in the natural products at the 8- and/or 14-positions were removed.[5] As shown in Scheme 1, two types of MS Core (**8** and **9**), which correspond to macrosphelides A−C and E−G, respectively, are mutually related as C3-epimeric isomers. Both MS Cores, including their nonnatural enantiomers (**ent-8** and **ent-9**), contain three stereogenic centers, and they can all be derived from a sole chiral building block, methyl 3-hydroxybutyrate (**7**). The availability of both enantiomers of **7** makes possible the synthesis of all four MS Cores by a common synthetic pathway. The bioactivities of the MS Cores could then be determined and compared with those of the natural products and the role of the oxygen substituents in the natural products evaluated.

Based on this idea, we accomplished a highly efficient synthesis of MS Cores, and evaluated their inhibitory effects on the in vitro proliferation of tumor cells. In this paper, we describe in detail this synthetic study and the results of the cell-proliferation assay.

Results and Discussion

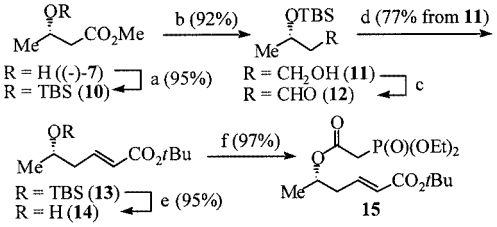
For the synthesis of the first target MS Core **8**, we designed a synthetic protocol in which three chiral molecules

[a] Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University,
2630 Sugitani, Toyama 930-0194, Japan
Fax: (internat:) + 81-76-434-5047
E-mail: nemotoh@ms.toyama-mpu.ac.jp
[b] Department of Pathogenic Biochemistry, Institute of Natural Medicine, Toyama Medical and Pharmaceutical University,
2630 Sugitani, Toyama 930-0194, Japan
Supporting information for this article is available on the WWW under http://www.eurjoc.org or from the author.



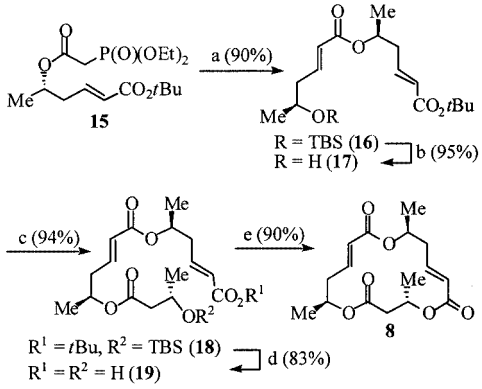
Scheme 1

derived from (−)-**7** were connected by sequential (*E*)-selective Horner−Emmons olefination, dehydrative esterification, and finally Yamaguchi's macrolactonization.[6] Commercially available methyl (*S*)-3-hydroxybutyrate [(−)-**7**] was converted into the known aldehyde **12** in three steps according to the reported procedure.[7] Horner−Emmons reaction and subsequent desilylation afforded the (*E*)-olefinic alcohol **14** as the sole geometric isomer, which was subjected to esterification with phosphonoacetic acid to furnish the second Horner−Emmons substrate **15** in good yields (Scheme 2).



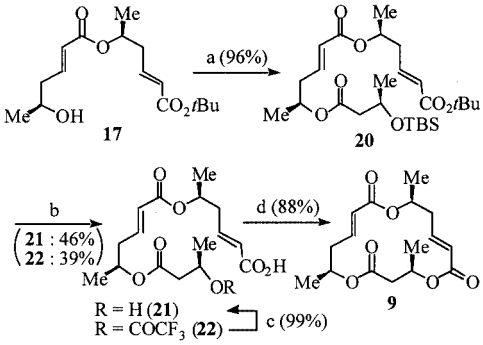
Scheme 2. Reagents and conditions: (a) TBSCl, Et_3N, cat. DMAP, CH_2Cl_2, room temp., 13 h; (b) DIBAL, CH_2Cl_2, room temp., 2 h; (c) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78 °C then 0 °C, 1 h; (d) *tert*-butyl diethylphosphonoacetate, DBU, LiCl, MeCN, room temp., 1 h; (e) TBAF, THF, room temp., 6 h; (f) diethylphosphonoacetic acid, DCC, cat. DMAP, CH_2Cl_2, room temp., 0.5 h

Reaction of the phosphonate **15** with the chiral aldehyde **12** yielded the (*E*)-olefin **16** stereoselectively. After desilylation, the resulting alcohol **17** was allowed to react with the chiral carboxylic acid prepared from **10** in the presence of DCC and DMAP to afford the triester **18**, which possessed a 16-atom chain and the three stereogenic centers of MS Core **8**. Concurrent removal of the TBS and *tert*-butyl groups of **18** was performed with thioanisole/TFA[8] to give the seco-acid **19** in 83% yield. Finally, macrocyclization of **19** was carried out using Yamaguchi's macrolactonization protocol,[6] and the synthesis of the target MS Core **8** was accomplished in 11 steps and 37.2% overall yield, starting from (−)-**7** (Scheme 3). Satisfactory spectroscopic data were obtained, and the structure of **8** was confirmed by X-ray crystallographic analysis.[9]



Scheme 3. Reagents and conditions: (a) **12**, DBU, LiCl, MeCN, room temp., 0.5 h; (b) AcOH/THF/H_2O (3:1:1), room temp., 3 d; (c) (*S*)-3-(*tert*-butyldimethylsilyloxy)butyric acid, DCC, cat. DMAP, CH_2Cl_2, room temp., 3 h; (d) thioanisole, TFA, CH_2Cl_2, room temp., 1 h; (e) 2,4,6-trichlorobenzoyl chloride, Et_3N, toluene, room temp., 1 h, then the reaction mixture was added to DMAP in toluene, 80 °C, 2 h

Another MS Core **9** (E−G series) was synthesized by replacing the (*S*) chiral carboxylic acid used in the synthesis of **18** above with the (*R*) enantiomer, as shown in Scheme 4.



Scheme 4. Reagents and conditions: (a) (*R*)-3-(*tert*-butyldimethylsilyloxy)butyric acid, DCC, cat. DMAP, CH_2Cl_2, room temp., 3 h; (b) thioanisole, TFA, CH_2Cl_2, room temp., 1 h; (c) $NaHCO_3$, THF/H_2O, room temp., 1 h; (d) 2,4,6-trichlorobenzoyl chloride, Et_3N, toluene, room temp., 1 h, then the reaction mixture was added to DMAP in toluene, 80 °C, 3 h

In this case, deprotection of **20** using TFA gave rise to a considerable amount of the trifluoroacetate **22**, which was readily converted into the requisite hydroxy acid **21** by a mild alkaline saponification in almost quantitative yield. The macrolactonization proceeded under the same conditions as used for the synthesis of **8** to furnish MS Core **9** in good yield. The nonnatural enantiomers (**ent-8** and **ent-9**) were also synthesized according to the above schemes. Thus, efficient synthetic routes for the target four MS Cores have been satisfactorily established.

With the synthetic MS Cores in hand, we focused our attention on their biological activities and compared these with the biological activities of the natural macrosphelides. To evaluate the antiproliferative activity, cultured murine colon 26-L5 adenocarcinoma cells (colon 26-L5) were used, and the activity was determined in vitro by the WST-1 cytotoxicity assay.[10] The compounds examined, three MS Cores (**8**, **ent-8**, and **9**), two natural macrosphelides (A and B),[11] and the diketone **23**[3a] synthesized from macrosphelide B by PDC oxidation, are listed in Figure 2. As shown in Figure 3 (A), the synthesized MS Cores exhibited a slight antiproliferative effect at a concentration of 10μM. However, at higher concentrations, instead of suppressing tumor cell growth, cell proliferation was significantly enhanced. Future characterization of this enhancement will provide a novel insight into the mode of action of synthesized MS Cores. On the other hand, natural macrosphelide B and the diketone **23** showed direct cytotoxicity against colon 26-L5 cells, although macrosphelide A did not show such an effect (Figure 3, B).

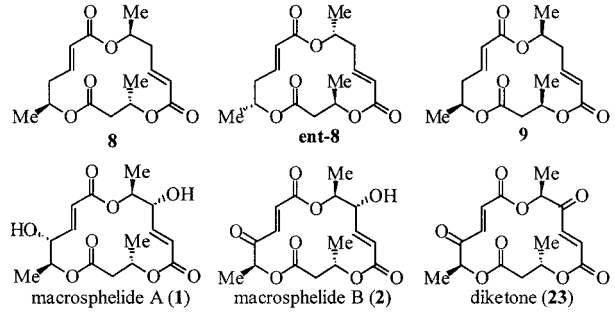


Figure 2. Chemical structures of macrosphelides subjected to the bioassay

These results imply that the oxygen functionalities at the 8- and/or 14-position(s) of natural products play an important role in the inhibition of tumor cell growth, at least against colon 26-L5 cells. Although the MS Cores synthesized in this study did not exhibit any positive bioactivities in themselves, the results suggest that the introduction of oxygen functions into the core compounds might provide macrosphelide-like derivatives.

Conclusions

In summary, we have described the concise and highly efficient synthesis of MS Cores and compared their inhibitory effect on colon 26-L5 cell proliferation with that of the natural macrosphelides. The study described herein suggests that the hydroxy and oxo groups on the macrosphelide skeleton are important for their biological activity. In order to clarify these substituent effects, more extensive studies on the structure-activity relationships and conformational analysis of each compound are required, and are currently being investigated.

Experimental Section

General Remarks: All nonaqueous reactions were carried out under argon. Reagents were purchased from commercial sources and used as received. Anhydrous solvents were obtained from commercial sources or prepared by distillation from CaH_2 or P_2O_5. ^1H and ^{13}C NMR spectra were obtained with a Varian Gemini 300 (300 MHz for ^1H and 75.46 MHz for ^{13}C) instrument or a Varian UNITY plus 500 (500 MHz for ^1H and 125 MHz for ^{13}C) instrument using tetramethylsilane or chloroform as the internal reference. Mass spectra were measured with a JEOL D-200 or a JEOL AX 505 mass spectrometer using the electron impact (EI, 70 eV) ionization method. IR spectra were recorded with a Perkin−Elmer 1600 spectrometer. The optical rotations were determined with a JASCO DIP-1000 instrument. Melting points were measured with a Yanagimoto micro melting point apparatus and are uncorrected. Column chromatography was carried out by employing Cica Silica Gel 60 N (spherical, neutral, 40−50 μm or 63−210 μm). Compounds **10**−**12** were prepared according to a reported method.[7]

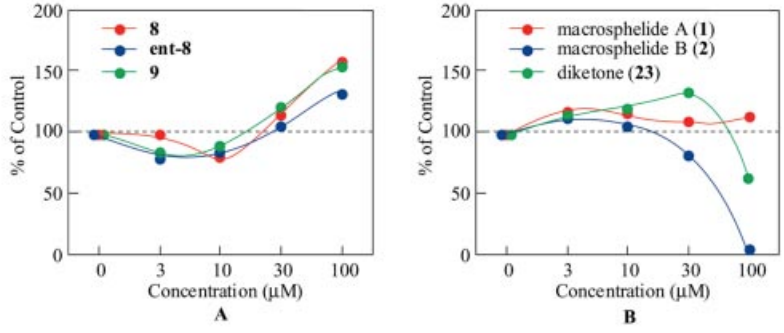


Figure 3. Effect of macrosphelides on the proliferation of Colon 26-L5 cells; proliferation was assessed by measuring the absorbance of the culture at 450 nm after treatment with WST-1

(+)-*tert*-Butyl 5-(*tert*-Butyldimethylsilyloxy)hex-2-enoate (13): Oxalyl chloride (0.88 mL, 10.08 mmol) was added to a stirred solution of dimethyl sulfoxide (1.08 mL, 15.12 mmol) in CH_2Cl_2 (28 mL) at −78 °C under Ar. After continuous stirring for 10 min, alcohol **11** (1.03 g, 5.04 mmol) was added dropwise to the solution. After 1 h, Et_3N (4.29 mL, 35.3 mmol) was added and the mixture was warmed to 0 °C, and stirring was continued for 15 min. The mixture was diluted with diethyl ether and water, and then extracted with diethyl ether. The extracts were combined and washed successively with 10% HCl, satd. $NaHCO_3$ and brine, and dried with $MgSO_4$. The solvent was evaporated to give the aldehyde **12** in almost pure form. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.76 (dd, *J* = 2.2, 2.7 Hz, 1 H), 4.33 (m, 1 H), 2.53 (ddd, *J* = 2.7, 6.9, 16 Hz, 1 H), 2.44 (ddd, *J* = 2.2, 4.9, 16 Hz, 1 H), 1.21 (d, *J* = 6.3 Hz, 3 H), 0.85 (s, 9 H), 0.05 (s, 3 H), 0.04 (s, 3 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 202.1, 64.7, 53.2, 26.0, 24.4, 18.2, −4.1, −4.6 ppm. *tert*-Butyl diethylphosphonoacetate (1.31 mL, 5.55 mmol) and DBU (0.693 mL, 5.04 mmol) were added to a stirred suspension of LiCl (256 mg, 6.05 mmol) in MeCN at 0 °C under Ar. After stirring for 0.5 h, the crude aldehyde **12** was added to the mixture. After stirring at room temperature for a further 1 h, the solvent was evaporated. A solution of the residue in diethyl ether was washed successively with 10% HCl, satd. $NaHCO_3$ and brine, and dried with $MgSO_4$. The residue resulting from evaporation of the solvent was submitted to chromatography on silica gel with hexane/EtOAc (50:1) to give the conjugated ester **13** (1.18 g, 77% from **11**) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.82 (dt, *J* = 7.7, 15 Hz, 1 H), 5.73 (dt, *J* = 1.4, 15 Hz, 1 H), 3.89 (m, 1 H), 2.26 (m, 2 H), 1.47 (s, 9 H), 1.15 (d, *J* = 6.0 Hz, 3 H), 0.87 (s, 9 H), 0.04 (s, 6 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 165.8, 144.8, 125.0, 80.2, 68.0, 42.6, 28.4, 26.1, 24.1, 18.40, −4.2, −4.5 ppm. IR (neat): $\tilde{\nu}$ = 1715, 1650 cm^{-1}. MS (EI): *m*/*z* = 187 [M$^+$ − 57]. HRMS (EI): calcd for $C_8H_{15}O_3Si$ 187.0860 [M$^+$ − 57]; found 187.0835. $[\alpha]_D^{25}$ = +11.9 (*c* = 1.27, $CHCl_3$).

(+)-*tert*-Butyl 5-Hydroxyhex-2-enoate (14): A 1 M solution of tetrabutylammonium fluoride in THF (7.03 mL, 7.03 mmol) was added to a stirred solution of the silyl ether **13** (1.76 g, 5.86 mmol) in THF (12 mL) at room temperature under Ar and the mixture was stirred at room temperature for 6 h . The solvent was evaporated and the resulting residue dissolved in diethyl ether was washed with water and brine, and dried with $MgSO_4$. Evaporation of the solvent left a residue which was submitted to chromatography on silica gel with hexane/EtOAc (4:1) to give the hydroxy ester **14** (1.02 g, 95%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.83 (dt, *J* = 7.4, 15 Hz, 1 H), 5.71 (dt, *J* = 1.4, 15 Hz, 1 H), 3.94 (m, 1 H), 2.32 (ddd, *J* = 1.4, 6.3, 7.4 Hz, 2 H), 1.47 (s, 9 H), 1.22 (d, *J* = 6.3 Hz, 3 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 165.8, 143.7, 125.7, 80.5, 66.9, 42.0, 28.4, 23.4 ppm. IR (neat): $\tilde{\nu}$ = 3400, 1710, 1650 cm^{-1}. MS (EI): *m*/*z* = 142 [M$^+$ − 44], 130 [M$^+$ − 56]. HRMS (EI): calcd. for $C_8H_{14}O_2$ 142.0994 [M$^+$ − 44]; found 142.0992; calcd. for $C_6H_{10}O_3$: 130.0630 [M$^+$ − 56]; found 130.0623. $[\alpha]_D^{26}$ = +10.2 (*c* = 1.17, $CHCl_3$).

(−)-*tert*-Butyl 5-[2-(Diethoxyphosphoryl)acetoxy]hex-2-enoate (15): 1,3-Dicyclohexylcarbodiimide (DCC) (1.48 g, 7.14 mmol) was added to a stirred solution of the hydroxy ester **14** (0.95 g, 5.1 mmol), diethylphosphonoacetic acid (0.98 mL, 6.12 mmol) and 4-dimethylaminopyridine (62 mg, 0.51 mmol) in CH_2Cl_2 (50 mL) at 0 °C under Ar. After continuous stirring at room temperature for 0.5 h, the solvent was evaporated to leave a residue that was triturated with diethyl ether and filtered through Celite. Evaporation of the solvent, followed by chromatography on silica gel with hexane/EtOAc (1:1) afforded the phosphonoacetate ester **15** (1.78 g, 97%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.77 (dt, *J* = 7.5, 15 Hz, 1 H), 5.80 (d, *J* = 15 Hz, 1 H), 5.04 (m, 1 H), 4.17 (dq, *J* = 7.1, 8.1 Hz, 4 H), 2.95 (d, *J* = 21 Hz, 2 H), 2.45 (m, 2 H), 1.48 (s, 9 H), 1.35 (t, *J* = 7.1 Hz, 6 H), 1.27 (d, *J* = 6.3 Hz, 3 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 165.2, 165.1 (d, *J* = 6.1 Hz), 141.6, 126.0, 80.3, 70.7, 62.7 (d, *J* = 6.1 Hz), 38.1, 34.6 (d, *J* = 133 Hz), 28.2, 19.5, 16.5 (d, *J* = 6.1 Hz) ppm. IR (neat): $\tilde{\nu}$ = 1730, 1710, 1650 cm^{-1}. MS (EI): *m*/*z* = 364 [M$^+$]. HRMS (EI): calcd. for $C_{16}H_{29}O_7P$ 364.1651 [M$^+$]; found 364.1634. $[\alpha]_D^{25}$ = −8.56 (*c* = 1.32, $CHCl_3$).

(+)-4-(*tert*-Butoxycarbonyl)-1-methylbut-3-enyl 5-(*tert*-Butyldimethylsilyloxy)hex-2-enoate (16): Phosphonoester **15** (960 mg, 2.6 mmol), DBU (400 mg, 2.6 mmol) and finally the aldehyde **12** (450 mg, 2.2 mmol) were added to a stirred suspension of LiCl (112 mg, 2.6 mmol) in MeCN (30 mL) at 0 °C under Ar. After stirring at room temperature for a further 0.5 h, the solvent was evaporated. A solution of the residue in diethyl ether was washed successively with water, 10% HCl, satd. $NaHCO_3$ and brine, and dried with $MgSO_4$. The residue resulting from evaporation of the solvent was submitted to chromatography on silica gel with hexane/EtOAc (95:5) to give the silyl ether **16** (978 mg, 90%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.91 (dt, *J* = 7.5, 15 Hz, 1 H), 6.85 (dt, *J* = 7.5, 15 Hz, 1 H), 5.78 (dt, *J* = 1.4, 15 Hz, 2 H), 5.03 (m, 1 H), 3.90 (m, 1 H), 2.44 (m, 2 H), 2.29 (m, 2 H), 1.45 (s, 9 H), 1.24 (d, *J* = 6.3 Hz, 3 H), 1.14 (d, *J* = 6.0 Hz, 3 H), 0.86 (s, 9 H), 0.03 (s, 3 H), 0.02 (s, 3 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 165.7, 165.5, 146.4, 142.3, 125.9, 123.3, 80.4, 69.2, 67.8, 42.7, 38.5, 28.4, 26.1, 24.1, 19.9, 18.3, −4.2, −4.5 ppm. IR (neat): $\tilde{\nu}$ = 1710, 1650 cm^{-1}. MS (EI): *m*/*z* = 412 [M$^+$]. HRMS (EI): calcd. for $C_{22}H_{40}O_5Si$ 412.2646 [M$^+$]; found 412.2667. $[\alpha]_D^{24}$ = +5.92 (*c* = 1.07, $CHCl_3$).

(+)-4-(*tert*-Butoxycarbonyl)-1-methylbut-3-enyl 5-Hydroxyhex-2-enoate (17): TBS ether **16** (5.11 g, 12.4 mmol) was dissolved in AcOH/THF/H_2O (3:1:1; 248 mL). The solution was stirred at room temperature for 3 d and then extracted with $CHCl_3$. The combined extracts were washed with satd. $NaHCO_3$, dried with $MgSO_4$, and concentrated. The residue was submitted to chromatography on silica gel with hexane/EtOAc (2:1) to give the hydroxy ester **17** (3.5 g, 95%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.93 (dt, *J* = 7.4, 15 Hz, 1 H), 6.75 (dt, *J* = 7.5, 15 Hz, 1 H), 5.85 (d, *J* = 15 Hz, 1 H), 5.76 (d, *J* = 15 Hz, 1 H), 5.14 (m, 1 H), 3.94 (m, 1 H), 2.45 (m, 2 H), 2.33 (m, 2 H), 2.20 (br, 1 H), 1.45 (s, 9 H), 1.24 (d, *J* = 6.3 Hz, 3 H), 1.21 (d, *J* = 6.0 Hz, 3 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 165.7, 165.6, 145.4, 142.3, 126.0, 123.9, 80.5, 69.4, 66.8, 42.1, 38.5, 28.4, 23.5, 19.9 ppm. IR (neat): $\tilde{\nu}$ = 3400, 1710, 1650 cm^{-1}. MS (EI): *m*/*z* = 298 [M$^+$]. HRMS (EI): calcd. for $C_{16}H_{26}O_5$ 298.1781 [M$^+$]; found 298.1779. $[\alpha]_D^{26}$ = +5.57 (*c* = 0.86, $CHCl_3$).

(−)-4-(*tert*-Butoxycarbonyl)-1-methylbut-3-enyl 5-[3-(*tert*-Butyldimethylsilyloxy)butyryloxy]hex-2-enoate (18): 1,3-Dicyclohexylcarbodiimide (DCC) (55 mg, 0.265 mmol) was added to a stirred solution of the hydroxy ester **17** (50 mg, 0.17 mmol), (*S*)-3-(*tert*-butyldimethylsilyloxy)butyric acid (43 mg, 0.20 mmol), and 4-dimethylaminopyridine (2.0 mg, 0.017 mmol) in CH_2Cl_2 (2 mL) at 0 °C under Ar. After continuous stirring at room temperature for 3 h , the solvent was evaporated to leave a residue, which was triturated with diethyl ether and filtered through Celite. Evaporation of the solvent, followed by chromatography on silica gel with hexane/EtOAc (20:1) afforded the ester **18** (81 mg, 94%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.95 (dt, *J* = 7.5, 15 Hz, 1 H), 6.75 (dt, *J* = 7.7, 15 Hz, 1 H), 5.83 (d, *J* = 15 Hz, 1 H), 5.77 (d, *J* = 15 Hz, 1 H), 4.97 (m, 2 H), 4.23 (m, 1 H), 2.45 (m, 2 H), 2.33 (m,

2 H), 2.30 (m, 2 H), 1.46 (s, 9 H), 1.25 (d, J = 6.8 Hz, 3 H), 1.22 (d, J = 6.6 Hz, 3 H), 1.16 (d, J = 6.0 Hz, 3 H), 0.84 (s, 9 H), 0.05 (s, 3 H), 0.03 (s, 3 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.9, 165.5, 165.4, 143.7, 142.2, 126.0, 124.3, 80.5, 69.4, 69.2, 65.9, 45.2, 38.6, 38.5, 28.4, 26.0, 24.0, 19.9, 19.8, 18.2, −4.2, −4.6 ppm. IR (neat): ṽ = 1710, 1650 cm^{-1}. MS (EI): m/z = 498 [M$^+$]. HRMS (EI): calcd. for $C_{26}H_{46}O_7Si$ 498.3013 [M$^+$]; found 498.3012. $[\alpha]_D^{25}$ = −1.34 (c = 0.88, $CHCl_3$).

(−)-5-[5-(3-Hydroxybutyryloxy)hex-2-enoyloxy]hex-2-enoic Acid (19): Trifluoroacetic acid (2.5 mL) was added dropwise to a stirred solution of silyloxy ester **18** (500 mg, 1 mmol) in thioanisole (12.5 mL) and CH_2Cl_2 (12.5 mL) at 0 °C. The solution was stirred at room temperature for 1 h and then concentrated. Chromatography on silica gel with $CHCl_3$/MeOH (20:1) afforded the hydroxy acid **19** (273 mg, 83%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.97 (dt, J = 7.1, 15 Hz, 1 H), 6.85 (dt, J = 7.5, 15 Hz, 1 H), 5.88 (d, J = 15 Hz, 1 H), 5.83 (d, J = 15 Hz, 1 H), 5.12 (m, 2 H), 4.28 (m, 1 H), 2.44 (m, 6 H), 1.28 (d, J = 6.3 Hz, 3 H), 1.26 (d, J = 5.7 Hz, 3 H), 1.22 (d, J = 6.3 Hz, 3 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.2, 170.3, 165.5, 146.1, 143.9, 124.3, 123.7, 69.8, 69.2, 64.6, 43.2, 38.6, 38.8, 22.7, 20.1, 20.0 ppm. IR (neat): ṽ = 3400, 1710, 1650 cm^{-1}. MS (EI): m/z = 328 [M$^+$]. HRMS (EI): calcd. for $C_{16}H_{24}O_7$ 328.1522 [M$^+$]; found 328.1511. $[\alpha]_D^{25}$ = −8.31 (c = 1.43, $CHCl_3$).

Macrosphelide Core 8 (A−C Series): A solution of the hydroxy acid **19** (40 mg, 0.12 mmol) in toluene (10 mL) was treated with Et_3N (73 mg, 0.72 mmol) and 2,4,6-trichlorobenzoyl chloride (146 mg, 0.6 mmol) at room temperature. The solution was stirred at room temperature for 1 h, diluted with toluene (20 mL), and added to a solution of 4-dimethylaminopyridine (170 mg, 1.4 mmol) in toluene (10 mL) at 80 °C over 2 h. After an additional 3.5 h, the mixture was cooled to afford a white suspension. Addition of satd. $NaHCO_3$ gave a clear biphasic mixture. The layers were separated, the aqueous phase was extracted with EtOAc, and the combined organic solutions were dried with $MgSO_4$, filtered and concentrated. Chromatography on silica gel with hexane/EtOAc (1:1) furnished the macrosphelide core **8** (34 mg, 90%) as a colorless solid. M.p. 94−95 °C (colorless plates from hexane). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.88−6.74 (m, 2 H), 5.78 (dt, J = 1.5, 15 Hz, 2 H), 5.35 (m, 1 H), 5.13−5.01 (m, 2 H), 2.62−2.25 (m, 6 H), 1.34 (d, J = 6.3 Hz, 3 H), 1.29 (d, J = 6.3 Hz, 3 H), 1.26 (d, J = 6.3 Hz, 3 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.2, 165.3, 164.9, 144.6, 143.5, 125.0, 123.8, 70.3, 68.8, 67.9, 41.7, 39.6, 39.1, 21.3, 20.8, 20.4 ppm. IR (neat): ṽ = 1730, 1710, 1650 cm^{-1}. MS (EI): m/z = 310 [M$^+$]. HRMS (EI): calcd. for $C_{16}H_{22}O_6$ 310.1417 [M$^+$]; found 310.1404. $C_{16}H_{22}O_6$ (310.34): calcd. C 61.92, H 7.15, found C 61.91, H 7.00. $[\alpha]_D^{25}$ = +20.5 (c = 0.50, $CHCl_3$). The nonnatural enantiomer **ent-8** was also synthesized following the above procedure from methyl (*R*)-3-hydroxybutyrate as the starting material. The spectroscopic data of **ent-8** agreed with those of **8**. $[\alpha]_D^{25}$ = −19.9 (c = 0.50, $CHCl_3$).

(−)-4-(*tert*-Butoxycarbonyl)-1-methylbut-3-enyl 5-[3-(*tert*-Butyldimethylsilyloxy)butyryloxy]hex-2-enoate (20): According to the synthetic procedure used for the preparation of **18**, the alcohol **17** (405 mg, 1.34 mmol) and (*R*)-3-(*tert*-butyldimethylsilyloxy)butyric acid afforded the ester **20** (650 mg, 96%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.86 (dt, J = 7.4, 15 Hz, 1 H), 6.77 (dt, J = 7.4, 15 Hz, 1 H), 5.84 (d, J = 15 Hz, 1 H), 5.79 (d, J = 15 Hz, 1 H), 5.01 (m, 2 H), 4.23 (m, 1 H), 2.40 (m, 6 H), 1.47 (s, 9 H), 1.26 (d, J = 6.0 Hz, 3 H), 1.24 (d, J = 6.3 Hz, 3 H), 1.18 (d, J = 6.0 Hz, 3 H), 0.86 (s, 9 H), 0.06 (s, 3 H), 0.04 (s, 3 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.0, 165.6, 165.5, 143.8, 142.2, 126.0, 124.3, 80.5, 69.5, 69.2, 65.9, 45.2, 38.7, 38.5, 28.4, 26.0, 24.1, 20.0, 19.9, 18.3, −4.2, −4.6 ppm. IR (neat): ṽ = 1722, 1656 cm^{-1}. MS (EI): m/z = 498 [M$^+$]. HRMS (EI): calcd. for $C_{26}H_{46}O_7Si$ 498.3013 [M$^+$]; found 498.3012. $[\alpha]_D^{25}$ = −19.9 (c = 1.25, $CHCl_3$).

(−)-5-[5-(3-Hydroxybutyryloxy)hex-2-enoyloxy]hex-2-enoic Acid (21): According to the synthetic procedure used for the preparation of **19**, the silyloxy ester **20** (241 mg, 0.48 mmol) afforded the hydroxy acid **21** (79 mg, 46%) and the trifluoroacetate **22** (73 mg, 39%) as colorless oils. Compound **22** was converted into **21** by treatment with $NaHCO_3$ in THF/H_2O at room temperature for 1 h in quantitative yield. **21:** ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.99 (dt, J = 7.3, 16 Hz, 1 H), 6.87 (dt, J = 7.3, 16 Hz, 1 H), 5.81 (d, J = 16 Hz, 1 H), 5.78 (d, J = 16 Hz, 1 H), 5.00 (m, 2 H), 4.10 (m, 1 H), 2.40 (m, 6 H), 1.22 (d, J = 6.3 Hz, 3 H), 1.20 (d, J = 6.3 Hz, 3 H), 1.16 (d, J = 6.3 Hz, 3 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.2, 170.3, 165.5, 146.0, 143.9, 124.3, 123.8, 69.7, 69.3, 64.6, 43.2, 38.6, 38.6, 22.7, 20.1, 20.0 ppm. IR (neat): ṽ = 3455, 2978, 1713, 1656 cm^{-1}. MS (EI): m/z = 328 [M$^+$]. HRMS (EI): calcd. for $C_{16}H_{24}O_7$ 328.1522 [M$^+$]; found 328.1511. $[\alpha]_D^{25}$ = −34.6 (c = 1.45, $CHCl_3$). **22:** ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.02 (dt, J = 7.4, 15 Hz, 1 H), 6.85 (dt, J = 7.7, 15 Hz, 1 H), 5.90 (d, J = 15 Hz, 1 H), 5.85 (d, J = 15 Hz, 1 H), 5.49 (m, 1 H), 5.90 (m, 2 H), 2.76 (dd, J = 8.2, 16 Hz, 1 H), 2.50 (m, 5 H), 1.42 (d, J = 6.3 Hz, 3 H), 1.29 (d, J = 6.3 Hz, 3 H), 1.25 (d, J = 6.6 Hz, 3 H) ppm. MS (EI): m/z = 424 [M$^+$].

Macrosphelide Core 9 (E−G Series): According to the synthetic procedure used for the preparation of **8**, the hydroxy acid **21** (20 mg, 0.061 mmol) afforded the macrosphelide core **9** (16.6 mg, 88%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.93 (dt, J = 7.6, 16 Hz, 1 H), 6.78 (dt, J = 7.3, 16 Hz, 1 H), 5.79 (d, J = 16 Hz, 1 H), 5.78 (d, J = 16 Hz, 1 H), 5.28−5.20 (m, 1 H), 5.17−5.09 (m, 1 H), 5.02−4.99 (m, 1 H), 2.78−2.65 (m, 2 H), 2.55−2.43 (m, 2 H), 2.40−2.32 (m, 2 H), 1.42 (d, J = 6.3 Hz, 3 H), 1.36 (d, J = 6.4 Hz, 3 H), 1.26 (d, J = 6.3 Hz, 3 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 169.6, 165.2, 164.9, 143.9, 143.4, 124.5, 123.8, 70.7, 68.7, 67.2, 41.3, 38.2, 37.7, 20.4, 19.9, 19.5 ppm. IR (neat): ṽ = 1727, 1657 cm^{-1}. MS (EI): m/z = 310 [M$^+$]. HRMS (EI): calcd. for $C_{16}H_{22}O_6$: 310.1417 [M$^+$]; found 310.1404. $[\alpha]_D^{25}$ = +8.4 (c = 1.00, $CHCl_3$). The nonnatural enantiomer **ent-9** was also synthesized according to the above procedure. The spectroscopic data of **ent-9** agree with those of **9**. $[\alpha]_D^{25}$ = −11.4 (c = 0.86, $CHCl_3$).

Procedure for the Cell-Proliferation Assay: Tumor cells were cultured as follows. The murine colon 26-L5 adenocarcinoma cells (colon 26-L5) were maintained as monolayer cultures in an RPMI-1640 medium supplemented with 10% fetal calf serum, 0.1% $NaHCO_3$, and 2 mM glutamine. Next, the cells were incubated at 37 °C in a humidified atmosphere containing 5% CO_2 in air. The inhibitory effect of the test compounds on cell proliferation was determined by measuring the absorbance of the culture at 450 nm after treatment with WST-1 (DOJINDO). Namely, colon 26-L5 cells (1×10^4) were suspended in an RPMI medium (100 μL) containing 0.03% BSA, and seeded into each well of a 96-well culture plate, then pre-incubated for 24 h. Various concentrations of the test compounds, diluted with DMSO and then medium, were added to each well, and incubated for an additional 24 h. After addition of WST-1 solution (10 μL) and further incubation for 4 h, the amount of formazan formed was measured spectrophotometrically by the absorbance at 450 nm. Each assay was performed in triplicate for **8**, **ent-8**, and **9**, and in quadruplicate for macrosphelide A, B, and **23**.

X-ray Crystallographic Study: MS Core **8** was recrystallized from hexane to form a colorless platelet crystal ($C_{16}H_{22}O_6$) with approximate dimensions of 0.40 × 0.20 × 0.07 mm [a primitive orthorhombic space group $P2_12_12_1$ with unit cell parameters a = 7.7195(3), b = 10.0492(4), c = 20.8979(8) Å, V = 1621.2(1) Å^3, Z = 4, $D_{calcd.}$ = 1.27 g·cm^{-3}]. The data were collected with a Rigaku RAXIS-RAPID Imaging Plate diffractometer with graphite-monochromated Mo-K_α radiation (λ = 0.71069 Å). Of the 19297 reflections that were collected, 2687 were unique (R_{int} = 0.038). The structure was solved by direct methods (SIR97), and the full-matrix least-squares refinement was based on 2685 observed reflections [$I \geq 3.00\ \sigma(I)$] and 199 variable parameters [R = 0.059, R_w = 0.093, $R1$ = 0.040 for 2135 $I > 2.0\ \sigma(I)$ data].

Supporting Information (see also footnote on the first page of this article): ^{1}H and ^{13}C NMR spectra for all new compounds and ORTEP drawing for MS Core **8**.

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