Absolute stereostructures of cell-adhesion inhibitors, macrosphelides C, E–G and I, produced by a *Periconia* species separated from an *Aplysia* sea hare

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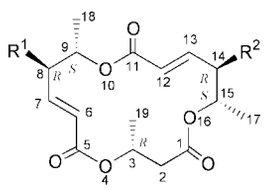
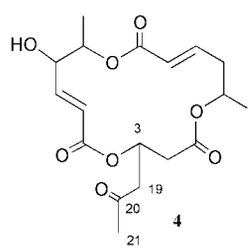
Macrosphelides E–I have been isolated, along with known macrosphelides A and C, from a strain of *Periconia byssoides* originally separated from the sea hare *Aplysia kurodai*, and the absolute stereostructures of macrosphelides E–G and I have been elucidated on the basis of spectroscopic analyses using 1D and 2D NMR techniques and some chemical transformations. In addition, the absolute configuration of macrosphelide C, previously undetermined, has been established by X-ray analysis and application of the modified Mosher method. Macrosphelides E–H inhibited the adhesion of human-leukaemia HL-60 cells to HUVEC.

Introduction

Based on the fact that some of the bioactive materials isolated from marine animals have been produced by bacteria, we have chosen to seek new antitumour metabolites from microorganisms inhabiting the marine environment.[1–3] As part of this program, we have made a search for antitumour compounds from a strain of *Periconia byssoides* OUPS-N133 which was separated from the sea hare *Aplysia kurodai*, and isolated pericosine A as an antitumour compound by bioassay-directed fractionation (cytotoxicity against P388 lymphocytic leukaemia cells) from a culture broth of this fungal strain.[4] In the course of this experiment, we have found five new 16-membered macrolides, macrosphelides E–I **1**–**5**, along with known macrosphelides A **6** and C **7**, from non-cytotoxic fractions from column chromatography. Omura and co-workers have found that macrosphelides A **6**–D, produced by *Microsphaeropsis* sp., potently inhibit cell–cell adhesion,[5] and reported the absolute stereostructures and total synthesis of macrosphelides A **6** and B,[6,7] and the planar structures of macrosphelides C, D, J and K.[8,9] Arthritis and metastasis have been found to be associated with cell-adhesion molecules.[10,11] This has evoked wide interest in inhibitors of cell adhesion because of the potential for the treatment of inflammation and tumour metastasis. Macrosphelides E **1**–H **4** isolated in this experiment also inhibited the adhesion of human-leukaemia HL-60 cells to human-umbilical-vein endothelial cells (HUVEC). We describe herein the absolute stereostructures[12] of macrosphelides E **1**–G **3**, I **5** and C **7**, and the planar structure of macrosphelide H **4** in addition to their inhibition of cell adhesion. The absolute stereostructure of **1** and the planar structures of **2**–**4** have been briefly reported in a preliminary form.[4]



1 $R^1 - R^2 - OH$
2 $R^1 - H$, $R^2 - OH$
3 $R^1 - OH$, $R^2 - H$



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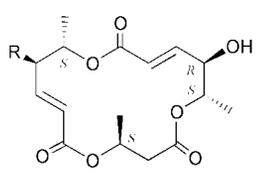
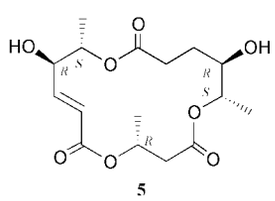


6 R – OH
7 R – H



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Results and discussion

The fungal strain was cultured at 27 °C for 4 weeks in a medium containing 1% malt extract, 1% glucose and 0.05% peptone in artificial seawater adjusted to pH 7.5. The AcOEt extract of the culture filtrate was purified by fractionation employing a combination of Sephadex LH-20 and silica gel column chromatography and high-performance liquid chromatography (HPLC) to afford macrosphelides A **6**, C **7**, and E **1**–I **5**. The known macrosphelides A **6** and C **7** were identified by comparison of spectral data with published values.[6,8]

Macrosphelide E **1** had the molecular formula $C_{16}H_{22}O_8$ established by the $[M + H]^+$ peak of **1** in high-resolution electron-impact mass spectrometry (HREIMS). Its IR spectrum exhibited bands at 3438, 1732, 1720, 1692, 1665 and 1648 cm^{-1}, characteristic of hydroxy and carbonyl groups, and a double bond. A close inspection of the ^1H and ^{13}C NMR spectra of **1** (Table 1) by DEPT and ^1H–^{13}C correlation spectroscopy (COSY) experiments revealed the presence of three secondary methyl (C-17, C-18 and C-19), one sp^3-hybridized methylene (C-2), five oxygen-bearing sp^3-methines (C-3, C-8, C-9, C-14 and C-15), two 1,2-disubstituted double bonds (C-6, C-7, C-12 and C-13) and three ester/lactone carbonyl groups (C-1, C-5 and C-11). The ^1H–^1H COSY analysis of **1** led to three partial structural units as shown by bold-faced lines in Fig. 1, which were supported by HMBC correlations (Table 1). The *E*-geometry of both the Δ^6- and Δ^{12}-double

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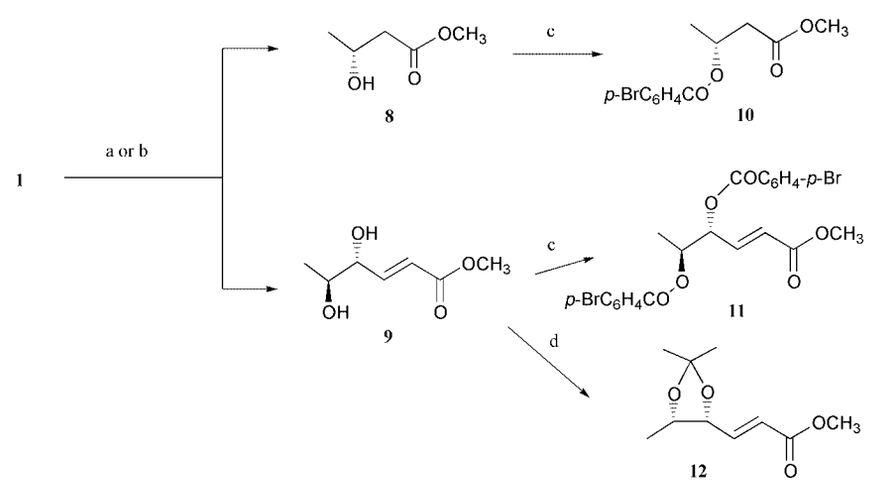
Table 1 ^{1}H and ^{13}C NMR data of macrosphelide E **1** in $CDCl_3$

Position	δ_H [a]	*J*/Hz	^{1}H–^{1}H COSY	δ_C	HMBC (C)
1				170.76 (q) [b]	
2 A	2.52 dd	15.6 (2B), 6.5 (3)	3	40.22 (s)	1, 3, 19
B	2.7 dd	15.6 (2A), 3.5 (3)	3		1, 3, 9
3	5.22 quint d	6.5 (2A, 19), 3.5 (2B)	2	66.83 (t)	1, 2A, 2B, 5, 19
5				166.48 (q)	
6	6.08 dd	15.7 (7), 1.5 (8)	6	122.37 (t)	5, 7, 8
7	6.97 dd	15.7 (6), 4.2 (8)	6, 8	145.99 (t)	5, 6, 8, 9
8	4.30 br s		7, 9, 8-OH	74.18 (t)	6, 7, 9, 18
9	5.04 qd	6.8 (18), 2.0 (8)	8, 18	75.02 (t)	7, 8, 18, 11
11				165.39 (q)	
12	5.97 dd	15.6 (13), 1.5 (14)	13	122.77 (t)	11, 13, 14
13	6.77 dd	15.6 (12), 6.9 (14)	12, 14	145.53 (t)	11, 12, 14, 15
14	4.09 dd	6.9 (13), 5.3 (15)	13, 15, 14-OH	73.35 (t)	12, 13, 15, 17
15	4.89 qd	6.5 (17), 5.3 (14)	14, 17	74.38 (t)	1, 13, 14, 17
17	1.23 d	6.4 (15)	15	17.29 (p)	14, 15
18	1.37 d	6.8 (9)	9	17.63 (p)	8, 9
19	1.33 d	6.5 (3)	3	19.32 (p)	2, 3
8-OH	3.88 br s		8		
14-OH	3.72 br s		14		

[a] ^{1}H Chemical-shift values (δ/ppm from $SiMe_4$) followed by multiplicity and then the coupling constant. Figures in parentheses indicate the proton coupling with that position. [b] Letters p, s, t and q, in parentheses, indicate, respectively, primary, secondary, tertiary and quaternary carbons, assigned by DEPT.



Scheme 1 *Reaction conditions*: (a) i. K_2CO_3, ii. HCl, iii. CH_2N_2; (b) MeOH, H_2SO_4; (c) *p*-BrC_6H_4COCl, pyridine; (d) $(CH_3)_2C(OCH_3)_2$, PPTS.

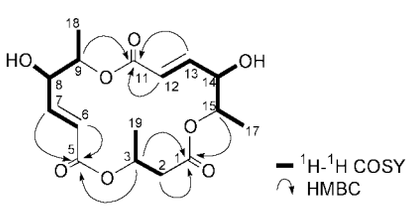


Fig. 1 Selected ^{1}H–^{1}H COSY and HMBC correlations in macrosphelide E **1**.

bonds was deduced from the coupling constants ($J_{6,7}$ 15.7 Hz and $J_{12,13}$ 15.6 Hz) of the olefinic protons. The connection of these three units and the remaining ester moiety was determined on the basis of the key HMBC correlations summarized in Fig. 1, and the planar structure of **1** was elucidated.

Since the stereochemistry of **1** could not be deduced from NOESY experiments, compound **1** was degraded to the methyl esters of the constituent carboxylic acids. Alkaline hydrolysis of **1** followed by treatment with diazomethane or acid-catalyzed methanolysis of **1** in MeOH gave only two products, methyl esters (respectively **8** and **9**) of 3-hydroxybutyric acid and 4,5-dihydroxyhex-2*E*-enoic acid, which were isolated as *p*-bromobenzoates (respectively **10** and **11**) because of the volatility of **8** (Scheme 1). This fact implied that the two dihydroxycarboxylic acid moieties of **1** have the same stereochemistry. In the acetonide **12** derived from the resulting ester **9**, the proton signals of the two isopropylidene methyl groups appeared with markedly different shifts (δ_H 1.38 and 1.52),[13] and NOEs were observed from one of the isopropylidene methyl groups (δ_H 1.38) to both H-4 and H-5 and from the other methyl group to H-3. From this evidence compound **12** was deduced to be the *anti*-acetonide and identified by comparison with published data.[14] The absolute configuration could not been determined because of a vanishingly small value of the published specific optical rotation ($[\alpha]_D$ -0.55×10^{-1} dm^3 mol^{-1} cm^{-1})[14] and the small amount of the acetonide. On the other hand, bis-*p*-bromobenzoate derivative **11** from the methyl ester **9** exhibited a weak single curve at 240 nm (Δ +1.26) in the CD spectrum. As reported by Harada, *syn*-1,2-acyclic diol dibenzoates exhibit typical exciton split Cotton effects in the CD spectra, from which their absolute configuration can be determined,[15] whereas the absolute configuration of *anti*-dibenzoates is difficult to deduce from the CD spectra because of a weak single Cotton effect.[16] Therefore, the CD data of **11** did not define its absolute configuration, though it was assumed to be 4*R*,5*S* on the basis of the single Cotton effect. The other *p*-bromobenzoate **10**, derived from **8**, was identified as methyl (3*R*)-3-(*p*-bromobenzoyloxy)butyrate by comparison of spectral data including CD spectra with the synthetic compound prepared from commercial methyl (3*R*)-3-hydroxybutyrate, implying that the chirality of the 3-hydroxybutyric acid moiety in **1** is *R*.

Table 2 ^{1}H and ^{13}C NMR data of macrosphelide F **2** in $CDCl_3$

Position	δ_H [a]	*J*/Hz	^{1}H–^{1}H COSY	δ_C	HMBC (C)
1				170.78 (q) [b]	
2 A	2.59 dd	15.8 (2B), 7.8 (3)	2B, 3	40.72 (s)	1, 3, 19
B	2.67 dd	15.8 (2A), 3.3 (3)	2A, 3		1, 3, 19
3	5.31 dqd	7.8 (2A), 6.5 (19), 3.3 (2B)	2	66.47 (t)	1, 2A, 2B, 5, 19
5				165.11 (q)	
6	5.80 dt	15.7 (7), 1.3 (8A, 8B)	7	124.55 (t)	5, 7, 8B
7	6.90 ddd	15.7 (6), 7.5 (8A), 6.8 (8B)	6, 8A, 8B	143.41 (t)	5, 6, 8A, 8B, 9
8 A	2.40 dddd	14.6 (8B), 7.5 (7), 6.8 (9), 1.3 (6)	7, 8B, 9	37.73 (s)	7, 9, 18
B	2.72 dddd	14.6 (8A), 6.8 (7), 4.8 (9), 1.3 (6)	7, 8A, 9		6, 7, 9, 18
9	5.15 quint d	6.8 (8A, 18), 4.8 (8B)	8A, 8B, 18	68.88 (t)	7, 8A, 18, 11
11				164.89 (q)	
12	6.10 dd	15.6 (13), 1.9 (14)	13	123.02 (t)	11, 13, 14
13	6.86 dd	15.6 (12), 4.2 (14)	12, 14	144.14 (t)	11, 12, 14, 15
14	4.22 br s		13, 15, 14-OH	73.56 (t)	12, 13, 15, 17
15	4.95 qd	6.6 (17), 3.9 (14)	14, 17	76.04 (t)	1, 13, 14, 17
17	1.32 d	6.6 (15)	15	17.49 (p)	14, 15
18	1.39 d	6.8 (9)	9	19.87 (p)	8A, 9
19	1.36 d	6.5 (3)	3	19.81 (p)	2, 3
14-OH	3.00 br s		14		

[a,b] As in Table 1.

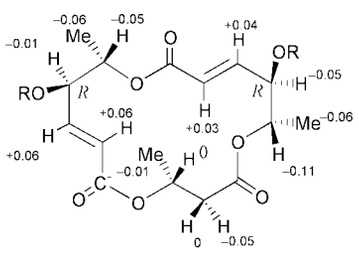
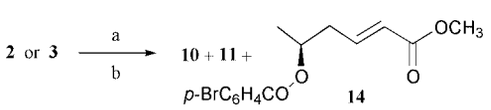


Fig. 2 ^{1}H Chemical-shift differences ($\Delta\delta = \delta_S - \delta_R$) between the (*R*)- and (*S*)-MTPA esters **13a** and **13b** of macrosphelide E **1**.

Since the chirality of the 4,5-dihydroxyhex-2*E*-enoic acid moiety in **1** could not be determined by CD, the modified Mosher method [17] was applied for determination of the absolute configuration of macrosphelide E **1**. The ^{1}H chemical-shift differences between the '(*R*)- and (*S*)-2-methoxy-2-phenyl-2-(trifluoromethyl)acetic acid' (MTPA) esters **13a** and **13b** of compound **1** are shown in Fig. 2. The result suggested 8*R* and 14*R* configurations, and hence allowed assignment of absolute stereostructure **1** with the 3*R*,8*R*,9*S*,14*R*,15*S* configuration to macrosphelide E. From this result, the absolute configuration of the bis-*p*-bromobenzoate derivative **11** was confirmed to be 4*R*,5*S*. Based on the above evidence, macrosphelide E **1** was found to be the C-3 stereoisomer of macrosphelide A **6**.[6,7]

Macrosphelide F **2** was assigned a molecular formula which contained one oxygen atom less than that of **1**. The general features of its UV, IR and NMR spectra closely resembled those of **1** except that the proton and carbon signals of one (C-8) of the hydroxymethines in **1** were replaced by those of a methylene in **2** (Tables 1 and 2). Analysis of ^{1}H–^{1}H COSY (7-H/8A-H and 8B-H, 9-H/8A-H and 8B-H, and 7-H/6-H) and HMBC (7-H/C-5, 3-H/C-5 and 9-H/C-11) correlations showed that the methylene is present at the 8-position and thus led to the planar structure of **2**. Methanolysis of **2** followed by treatment with *p*-bromobenzoyl chloride gave three *p*-bromobenzoates (Scheme 2). Two of them were identical with *p*-bromobenzoates **10** and **11** obtained from macrosphelide E **1**.



Scheme 2 *Reaction conditions*: (a) MeOH, H_2SO_4; (b) *p*-BrC_6H_4COCl, pyridine.

The third benzoate, **14**, was identified as methyl (5*S*)-5-(*p*-bromobenzoyloxy)hex-2*E*-enoate by comparison of spectral data including CD, with the enantiomer **15**, which was derived from methyl (5*R*)-5-hydroxyhex-2*E*-enoate [18,19] prepared from methyl (3*R*)-3-hydroxybutyrate by a modification of the procedure reported previously.[19,20] This evidence allowed assignment of absolute stereostructure **2** to macrosphelide F.

Macrosphelide G **3** had the same molecular formula as **2** as deduced from HREIMS. Its ^{1}H and ^{13}C NMR signals (Table 3) showed close correspondence with those of **2** (Table 2). ^{1}H–^{1}H COSY (8-H/7-H and 9-H, 7-H/6-H, 13-H/12-H, 14A-H and 14B-H, and 15-H/14A-H and 14B-H) and HMBC (7-H/C-5, 3-H/C-5, 9-H/C-11, 13-H/C-11 and 15-H/C-1) correlations in **3** implied that the C-8 methylene and C-14 methine in **2** change places with respect to each other in **3**. Thus, compound **3** is a positional isomer of **2**. In order to determine the absolute configuration for **3**, methanolysis of **3** followed by treatment of *p*-bromobenzoyl chloride was carried out using the same procedure as above with macrosphelide F **2**, to give the same three *p*-bromobenzoates as those obtained from **2** (Scheme 2). This evidence allowed assignment of absolute stereostructure **3** to macrosphelide G.

Macrosphelide H **4** had the molecular formula $C_{18}H_{24}O_8$ established by the $[M + H]^+$ peak in HREIMS. The general features of the ^{1}H and ^{13}C NMR spectra (Table 4) of **4** closely resembled those of **3** except that the signal for one of the methyl groups in **3** was replaced by that of an acetonyl group [δ_H 3.16, 3.19 (19-H_2); δ_C 45.24 (C-19); δ_C 205.78 (C-20); δ_H 2.22 (21-H_3), δ_C 30.57 (C-21)] in **4**. ^{1}H–^{1}H COSY (3-H/19-H, 2A-H and 2B-H) and HMBC (19-H/C-20 and 21-H/C-20) correlations (Table 4) revealed that the acetonyl group connects to the oxymethine at C-3, and hence the 3-hydroxybutyric acid moiety in **3** is replaced by a 3-hydroxy-5-oxohexanoic acid moiety in **4**. The position of the methylene groups in **4** was confirmed by ^{1}H–^{1}H COSY (13-H/12-H, 14A-H and 14B-H, and 15-H/14A-H and 14B-H) and HMBC (9-H/C-11, 13-H/C-11 and 15-H/C-1) correlations. Thus, the planar structure of **4** was elucidated.

Macrosphelide I **5** was assigned a molecular formula which contained two hydrogen atoms more than that of **1** as deduced from a molecular-ion peak in HREIMS. The general features of the ^{1}H and ^{13}C NMR spectra (Table 5) of **5** closely resembled those of **1** except that the signals for one of the disubstituted double bonds in **1** were replaced by those of an ethylene group [δ_H 2.36, 2.63 (H-12), δ_C 30.41 (C-12); δ_H 1.46, 1.71 (H-13), δ_C 27.45 (C-13)] in **5**. The analysis of ^{1}H–^{1}H COSY (13A-H/12-H, 13B-H and 14-H, and 14-H/15-H) and HMBC (9-H/C-11,

Table 3 ^{1}H and ^{13}C NMR data of macrosphelide G **3** in $CDCl_3$

Position	δ_H [a]	*J*/Hz	^{1}H–^{1}H COSY	δ_C	HMBC (C)
1				169 (q) [b]	
2 A	2.51 dd	15.7 (2B), 6.6 (3)	2B, 3	40 (s)	1, 3, 19
B	2.76 dd	15.7 (2A), 3.9 (3)	2A, 3		1, 3, 19
3	5.2 quint d	6.6 (2A, 19), 3.9 (2B)	2A, 2B	67.2 (t)	1, 2A, 2B, 5, 19
5				165 (q)	
6	6.11 dd	15.7 (7), 1.6 (8)	7	122 (t)	5, 7, 8
7	7.05 dd	15.7 (6), 3.9 (8)	6, 8	145 (t)	5, 6, 8, 9
8	4.36 br s		7, 8-OH, 9	75.3 (t)	6, 7, 9, 18
9	5.09 m		8, 18	76.4 (t)	7, 8, 18, 11
11				167 (q)	
12	5.80 dt	15.6 (13), 1.5 (14A, 14B)	13	123 (t)	11, 13, 14A, 14B
13	6.80 ddd	15.6 (12), 9.3 (14A), 6.2 (14B)	12, 14A, 14B	145 (t)	11, 12, 14B, 15
14 A	2.39 dtd	15.2 (14B), 9.3 (13, 15), 1.5 (12)	13, 14B, 15	38.3 (s)	12, 13, 15, 17
B	2.50 m		13, 14A, 15		12, 13, 17
15	5.08 m		14A, 14B, 17	70 (t)	1, 13, 14A, 17
17	1.26 d	6.4 (15)	15	20.3 (p)	14A, 14B, 15
18	1.41 d	6.8 (9)	9	17.9 (p)	8, 9
19	1.16 d	6.6 (3)	3	19.3 (p)	2, 3
8-OH	3.41 br s		8		

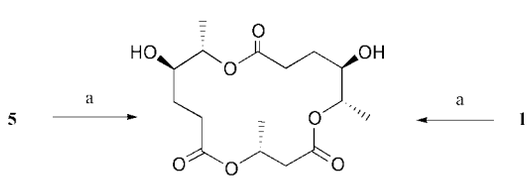
[a,b] As in Table 1.

Table 4 ^{1}H and ^{13}C NMR data of macrosphelide H **4** in $CDCl_3$

Position	δ_H [a]	*J*/Hz	^{1}H–^{1}H COSY	δ_C	HMBC (C)
1				169.22 (q) [b]	
2 A	2.60 dd	15.4 (2B), 3.5 (3)	2B, 3	36.75 (s)	1, 3, 19
B	2.93 dd	15.4 (2A), 4.6 (3)	2A, 3		1, 3, 19
3	5.52 m		2A, 19A, 19B	66.91 (t)	1, 2A, 2B, 5, 19
5				165.33 (q)	
6	6.10 dd	15.7 (7), 1.6 (8)	7	121.26 (t)	5, 7, 8
7	7.07 dd	15.7 (6), 3.9 (8)	6, 8	145.87 (t)	5, 6, 8, 9
8	4.38 br s		7, 9, 8-OH	75.34 (t)	6, 7, 9, 18
9	5.12 qd	6.8 (18), 1.6 (8)	8, 18	77.17 (t)	7, 8, 18, 11
11				167.31 (q)	
12	5.83 dt	15.6 (13), 1.3 (14A, 14B)	13	123.28 (t)	11, 13, 14A,
13	6.82 ddd	15.6 (12), 8.6 (14A), 6.5 (14B)	12, 14A, 14B	145.32 (t)	11, 12, 14B, 15
14 A	2.42 m		13, 14B, 15	38.29 (s)	12, 13, 15, 17
B	2.47 m		13, 14A, 15		12, 13, 17
15	5.05 m		14A, 14B, 17	70.03 (t)	1, 13, 14A, 17
17	1.29 d	6.2 (15)	15	20.37 (p)	14A, 14B, 15
18	1.43 d	6.8 (9)	9	17.89 (p)	8, 9
19 A	3.16 dd	18.2 (19B), 6.6 (3)	3, 19B	45.24 (s)	2, 3, 20, 21
B	3.19 dd	18.2 (19A), 7.3 (3)	3, 19A		
20				205.78 (q)	
21	2.22 s			30.57 (p)	19A, 19B, 20
8-OH	3.56 br s		8		

[a,b] As in Table 1.

13A-H/C-11 and 15-H/C-1) correlations (Table 5) clarified the ethylene moiety in **5** to be present at C-12 and C-13, and hence led to the planar structure of **5**. In order to determine the absolute configuration for **5**, it was hydrogenated in the presence of Pd–C to yield tetrahydro derivative **16**, identical with that derived by hydrogenation from macrosphelide E **1** (Scheme 3). Based on this evidence, the absolute stereostructure for **5** was elucidated.



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Scheme 3 *Reaction condition*: (a) H_2, Pd–C, MeOH, rt.

The planar structure of macrosphelide C **7** has already been reported by Omura and co-workers,[8] but the stereochemistry has remained undecided. Since the planar structure of **7** is identical with that of **2**, **7** is considered to be a stereoisomer of **2**. In order to determine the relative configuration of **7**, an X-ray crystal-structure analysis was carried out for a single crystal of **7** (obtained by recrystallization from hexane–CH_2Cl_2). The result obtained (Fig. 3) allowed assignment of the relative configuration of all the asymmetric centers and the conformation for **7**. The modified Mosher method was applied for determination of the absolute configuration of **7**. The ^{1}H chemical-shift differences between the (*R*)- and (*S*)-MTPA esters **17a** and **17b** of **7** are shown in Fig. 4, and the result suggested the *R* configuration for the asymmetric center at C-14. The above summarized evidence led to absolute stereostructure **7** for macrosphelide C.

Compounds **1**–**5** and derivative **16** were examined together with **6**, **7** and herbimycin A as standard samples in the adhesion-assay system using HL-60 cells and HUVEC, according to a modification of the method reported by Miki and co-workers.[21] As shown in Table 6, compounds **1**–**4** inhibited the adhesion of HL-60 cell to HUVEC more potently than herbimycin A, and compound **4** exhibited the most potent inhibitory activity. The cytotoxic activities of compounds **1**–**5**

Table 5 ^{1}H and ^{13}C NMR data of macrosphelide I **5** in $CDCl_3$

Position	δ_H [a]	*J*/Hz	^{1}H–^{1}H COSY	δ_C	HMBC (C)
1				168.6 (q) [b]	
2 A	2.64 dd	16.3 (2B), 10.3 (3)	2B, 3	41.76 (s)	1, 3, 19
B	2.70 dd	16.3 (2A), 3.1 (3)	2A, 3		1, 3, 19
3	5.65 dqd	10.3 (2A), 6.4 (19), 3.1 (2B)	2A, 2B	67.03 (t)	1, 2B, 5, 19
5				166.9 (q)	
6	6.24 dd	15.7 (7), 1.5 (8)	7	121.9 (t)	5, 7, 8
7	7.21 dd	15.7 (6), 4.2 (8)	6, 8	147.8 (t)	5, 6, 8, 9
8	4.29 br s		7, 9, 8-OH	74.84 (t)	6, 7, 9, 18
9	4.86 m		8, 18	78.33 (t)	7, 8, 18, 11
11				175.3 (q)	
12 A	2.36 dt	14.9 (12B), 4.8 (13A, 13B)	12B, 13A, 13B	30.41 (s)	11, 13A, 14
B	2.64 ddd	14.9 (12A), 11.8 (13B), 4.8 (13A)	12A, 13B		11, 13A, 13B
13 A	1.46 m		12A, 13B, 14	27.45 (s)	11, 12A, 12B, 15
B	1.71 dddd	16.6 (13A), 11.8 (12B), 4.8 (12A), 2.4 (14)	12B, 13A, 14		11, 12A, 14, 15
14	3.25 dt	11.2 (13A), 2.4 (13B, 15)	13A, 13B, 15	71.12 (t)	12A, 13B, 15, 17
15	4.85 m		14, 17	74.38 (t)	1, 13B, 14, 17
17	1.18 d	6.6 (15)	15	18.62 (p)	14, 15
18	1.47 d	6.9 (9)	9	12.67 (p)	8, 9
19	1.32 d	6.4 (3)	3	20.04 (p)	2, 3
8-OH	3.82 br s		8		
14-OH	2.53 br s				

[a,b] As in Table 1.

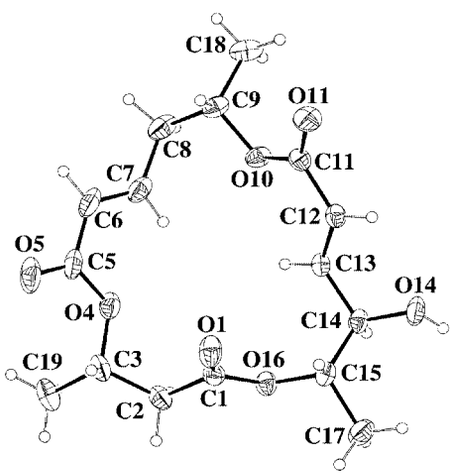
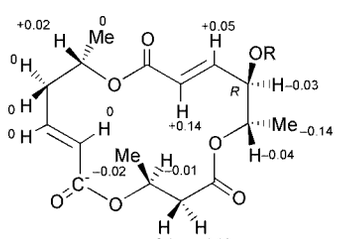


Fig. 3 X-Ray crystal structure for compound **7**. Displacement ellipsoids are drawn at the 40% probability level.



17a R = (*R*)-MTPA
17b R = (*S*)-MTPA

Fig. 4 ^{1}H Chemical-shift differences ($\Delta\delta = \delta_S - \delta_R$) between the (*R*)- and (*S*)-MTPA esters **17a** and **17b** of macrophelide C **7**.

were also examined against P388 lymphocytic leukaemia cells and HL-60 cells *in vitro*. Their ED_{50}-values were greater than 100 μg cm^{-3} against both cells except that the ED_{50}-value of compound **5** was 20 μg cm^{-3} against P388 cells.

Experimental

General procedures

Mps were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. 1D and 2D NMR spectra were recorded at 27 °C on a Varian UNITY INOVA-500 spectrometer, operating at 500 and 125 MHz for ^{1}H and ^{13}C, respectively, with TMS as internal reference. CD spectra were recorded on JASCO J-500A and J-820 polarimeters. The other procedures are the same as those reported previously.[1]

Table 6 Inhibitory activity of cell adhesion by macrosphelides

Compound	IC_{50} μ/M
Macrosphelide E **1**	19.5
F **2**	27.2
G **3**	22.5
H **4**	8.6
I **5**	>100
A **6** [a]	18.7
C **7** [a]	36.5
Derivative **16**	>100
Herbimycin A [a]	38.0

[a] Standard samples.

Culturing and isolation of metabolites

A strain of *Periconia byssoides* OUPS-N133 was initially isolated from the sea hare *Aplysia kurodai*, collected in the Osaka Bay of Japan in April, 1994. The sea hare was wiped with EtOH and its gastrointestinal tract applied to the surface of nutrient agar layered in a Petri dish. Serial transfers of one of the resulting colonies provided a pure strain of *P. byssoides*. The fungal strain was grown in a liquid medium (90 dm^3) containing 1% malt extract, 1% glucose and 0.05% peptone in artificial seawater adjusted to pH 7.5 for four weeks at 27 °C. The culture was filtered under suction and the mycelia collected were extracted thrice with MeOH. The combined extracts were evaporated *in vacuo* to give a mixture of crude metabolites (21.5 g), the CH_2Cl_2–MeOH (1 : 1)-soluble fraction of which exhibited cytotoxicity (ED_{50} >100 μg cm^{-3}). The culture filtrate was extracted thrice with AcOEt. The combined extracts were evaporated *in vacuo* to afford a mixture of crude metabolites (5.7 g), which exhibited cytotoxicity (ED_{50} 4.3 μg cm^{-3}). The AcOEt extract was passed through Sephadex LH-20, using CH_2Cl_2–MeOH (1 : 1) as the eluent.

The second fraction (3.5 g), in which the activity was concentrated, was chromatographed on a silica gel column with a CH_2Cl_2–MeOH gradient as the eluent. The MeOH–CH_2Cl_2 (2 : 98) and (5 : 95) eluates were collected as 2 fractions [Fr. 1 (126.6 mg) and Fr. 2 (54.1 mg)] and 2 fractions [Fr. 3 (315.7

mg) and Fr. 4 (254.6 mg)], respectively, all of which exhibited no cytotoxicity. Fr. 1 was purified by HPLC using MeOH–water (6 : 4) as the eluent to afford **2** (3.2 mg), **3** (6.7 mg), **4** (5.6 mg), and **7** (7.2 mg). Fr. 3 was purified by HPLC using MeOH–water (5 : 5) as the eluent to afford **1** (20.5 mg), **5** (11.1 mg) and **6** (0.8 mg).

Macrosphelide E 1. Obtained as plates, mp 105–107 °C (from hexane–CH_2Cl_2); $[\alpha]_D$ +56.8 (*c* 0.46 in EtOH); λ_{max} (EtOH)/nm 213 (log ε/dm^3 mol^{-1} cm^{-1} 4.18); ν_{max} (KBr)/cm^{-1} 3438 (OH), 1732, 1720, 1692 (ester), 1665 and 1648 (C=C); *m*/*z* (EI) 343 ([M + H]$^+$, 1.4%), 215 ([$C_{10}H_{15}O_5$]$^+$, 17.7), 111 ([$C_6H_7O_2$]$^+$, 100), 84 ([C_5H_8O]$^+$, 97.3) and 69 ([C_4H_5O]$^+$, 47.5) {*m*/*z* (HREI) Found: [M + H]$^+$, 343.1390. $C_{16}H_{23}O_8$ requires *m*/*z*, 343.1392}; CD λ (*c* 6.75 × 10^{-4} mol dm^{-3} in EtOH)/nm 289 ($\Delta\varepsilon$ 0), 248 (−0.59), 238 (0) and 227 (+1.70). ^{1}H and ^{13}C NMR data are listed in Table 1.

Macrosphelide F 2. Obtained as an oil, $[\alpha]_D$ +23.3 (*c* 0.09 in EtOH); λ_{max} (EtOH)/nm 216 (log ε/dm^3 mol^{-1} cm^{-1} 4.15); ν_{max} (liquid)/cm^{-1} 3445 (OH), 1720 (ester), 1658 and 1651 (C=C); *m*/*z* (EI) 327 ([M + H]$^+$, 0.8%), 111 ([$C_6H_7O_2$]$^+$, 100), 84 ([C_5H_8O]$^+$, 68.5) and 69 ([C_4H_5O]$^+$, 50.2) [*m*/*z* (HREI) Found: [M + H]$^+$, 327.1461. $C_{16}H_{23}O_7$ requires *m*/*z*, 327.1442]; CD λ (*c* 1.66 × 10^{-3} mol dm^{-3} in EtOH)/nm 291 ($\Delta\varepsilon$ 0), 240 (−3.18), 225 (0) and 213 (+8.72). ^{1}H and ^{13}C NMR data are listed in Table 2.

Macrosphelide G 3. Obtained as an oil, $[\alpha]_D$ +66.7 (*c* 0.48 in EtOH); λ_{max} (EtOH)/nm 217 (log ε/dm^3 mol^{-1} cm^{-1} 4.17); ν_{max} (liquid)/cm^{-1} 3443 (OH), 1718 (ester), 1658 and 1648 (C=C); *m*/*z* (EI) 326 (M$^+$, 1.8%), 111 ([$C_6H_7O_2$]$^+$, 100), 84 ([C_5H_8O]$^+$, 87.3) and 69 ([C_4H_5O]$^+$, 50.5) [*m*/*z* (HREI) Found: M$^+$, 326.1362. $C_{16}H_{23}O_8$ requires *M*, 326.1364]; CD λ (*c* 4.65 × 10^{-4} mol dm^{-3} in EtOH)/nm 308 ($\Delta\varepsilon$ 0), 235 (−2.37), 219 (0) and 215 (+0.92). ^{1}H and ^{13}C NMR data are listed in Table 3.

Macrosphelide H 4. Obtained as an oil, $[\alpha]_D$ +41.7 (*c* 0.22 in EtOH); λ_{max} (EtOH)/nm 214 (log ε/dm^3 mol^{-1} cm^{-1} 4.19); ν_{max} (liquid)/cm^{-1} 3452 (OH), 1724 (ester), 1661 and 1648 (C=C); *m*/*z* (EI) 369 ([M + H]$^+$, 1.8%), 111 ([$C_6H_7O_2$]$^+$, 100), 84 ([C_5H_8O]$^+$, 35.3) and 69 ([C_4H_5O]$^+$, 10.7) [*m*/*z* (HREI) Found: [M + H]$^+$, 369.1540. $C_{18}H_{25}O_8$ requires *m*/*z*, 369.1547]; CD λ (*c* 6.75 × 10^{-4} mol dm^{-3} in EtOH)/nm 311 ($\Delta\varepsilon$ 0), 239 (−1.07), 224 (0) and 220 (+0.54). ^{1}H and ^{13}C NMR data are listed in Table 4.

Macrosphelide I 5. Obtained as an oil, $[\alpha]_D$ +10.3 (*c* 0.31 in EtOH); ν_{max} (EtOH)/nm 215 (log ε/dm^3 mol^{-1} cm^{-1} 3.76); ν_{max} (liquid)/cm^{-1} 3436 (OH), 1718 (ester) and 1650 (C=C); *m*/*z* (EI) 345 ([M + H]$^+$, 1.1%), 217 ([$C_{10}H_{17}O_5$]$^+$, 66.3), 111 ([$C_6H_7O_2$]$^+$, 69.6), 84 ([C_5H_8O]$^+$, 100) and 69 ([C_4H_5O]$^+$, 62.2) [*m*/*z* (HREI) Found: [M + H]$^+$, 345.1550. $C_{16}H_{25}O_8$ requires *m*/*z*, 345.1547]; CD λ (*c* 5.22 × 10^{-4} mol dm^{-3} in EtOH)/nm 295 ($\Delta\varepsilon$ 0), 238 (−0.76), 224 (0) and 208 (+2.69). ^{1}H and ^{13}C NMR data are listed in Table 5.

Macrosphelide A 6. Obtained as needles, mp 139–141 °C (from hexane–CH_2Cl_2); $[\alpha]_D$ +78.8 (*c* 0.57 in EtOH); ν_{max} (KBr)/cm^{-1} 3432 (OH), 1718 (ester), 1668 and 1651 (C=C); *m*/*z* (EI) 343 ([M + H]$^+$, 1.8%), 215 ([$C_{10}H_{15}O_5$]$^+$, 10.7), 111 ([$C_6H_7O_2$]$^+$, 100), 84 ([C_5H_8O]$^+$, 85.7) and 69 ([C_4H_5O]$^+$, 25.8) [*m*/*z* (HREI) Found: [M + H]$^+$, 343.1390. $C_{16}H_{23}O_8$ requires *m*/*z*, 343.1392]; δ_H ($CDCl_3$) 1.33 (3H, d, *J* = 6.4 Hz, 19-H_3), 1.37 (3H, d, *J* = 6.4 Hz, 17-H_3), 1.46 (3H, d, *J* = 6.6 Hz, 18-H_3), 2.57 (1H, dd, *J* = 15.8, 3.7 Hz, 2-H_A), 2.62 (1H, dd, *J* = 15.8, 8.6 Hz, 2-H_B), 2.85 (1H, br s, 14-OH), 3.15 (1H, br s, 8-OH), 4.14 (1H, ddd, *J* = 6.2, 4.0, 1.5 Hz, 14-H), 4.23 (1H, ddd, *J* = 4.8, 4.0, 1.7 Hz, 8-H), 4.86 (1H, qd, *J* = 6.4, 6.2 Hz, 15-H), 4.96 (1H, qd, *J* = 6.6, 4.8 Hz, 9-H), 5.38 (1H, dqd, *J* = 8.6, 6.4, 3.7 Hz, 3-H), 6.04 (1H, dd, *J* = 15.7, 1.7 Hz, 6-H), 6.06 (1H, dd, *J* = 15.7, 1.5 Hz, 12-H), 6.87 (1H, dd, *J* = 15.7, 4.0 Hz, 13-H) and 6.88 (1H, dd, *J* = 15.7, 4.0 Hz, 7-H); CD λ (*c* 1.66 × 10^{-3} mol dm^{-3} in EtOH)/nm 291 ($\Delta\varepsilon$ 0), 247 (−1.07), 232 (0) and 218 (+3.67). ^{1}H and ^{13}C NMR data were identical with published values.[6]

Macrosphelide C 7. Obtained as needles, mp 156–158 °C (from hexane–CH_2Cl_2); $[\alpha]_D$ +46.3 (*c* 0.54 in EtOH); ν_{max} (KBr)/cm^{-1} 3460 (OH), 1731, 1705 (ester) and 1645 (C=C); *m*/*z* (HREI) Found: [M + H]$^+$, 327.1441. $C_{16}H_{23}O_7$ requires *m*/*z*, 327.1442; CD λ (*c* 1.66 × 10^{-3} mol dm^{-3} in EtOH)/nm 292 ($\Delta\varepsilon$ 0), 245 (−1.83), 233 (0) and 228 (+1.83). ^{1}H and ^{13}C NMR data were identical with published values.[8]

Acetonide 12 of degradation product 9 from macrosphelide E 1

A solution (2 cm^3) of 5% K_2CO_3 in water was added to a solution of macrosphelide E **1** (3.7 mg) in MeOH (0.05 cm^3), and the reaction mixture was left at room temperature overnight. The mixture was neutralized with 1 M HCl and extracted with diethyl ether, and then a solution of CH_2N_2 in diethyl ether was added to the ether layer. The mixture was left for 2 h at room temperature, and then the solvent was evaporated off under reduced pressure to afford a mixture of crude methyl esters (respectively **8** and **9**) of 3-hydroxybutyric acid and 4,5-dihydroxyhex-2*E*-enoic acid. To a solution of the mixture in CH_2Cl_2 (0.2 cm^3) were added 2,2-dimethoxypropane (0.3 cm^3) and pyridinum toluene-*p*-sulfonate (3.7 mg). After stirring of the mixture at room temperature for 1 h, the solvent was evaporated off under reduced pressure. The residue was purified by HPLC using MeOH–water (9 : 1) as the eluent to afford acetonide **12** (0.9 mg) as an oil.

Acetonide 12 [methyl (4*R*,5*S*)-4,5-isopropylidenedioxyhex-2*E*-enoate]. *m*/*z* (EIMS) 200 (M$^+$, 8.5%), 158 ($C_7H_{10}O_4$, 100.0) [*m*/*z* (HREI) Found: M$^+$, 200.1046. $C_{10}H_{16}O_4$ requires *M*, 200.1044]; δ_H ($CDCl_3$) 1.18 (3H, d, *J* = 6.4 Hz, 6-H_3), 1.38 (3H, s, acetonide CH_3), 1.52 (3H, s, acetonide CH_3), 3.76 (3H, s, $COOCH_3$), 4.43 (1H, quintet, *J* = 6.4 Hz, 5-H), 4.66 (1H, dd, *J* = 6.4, 5.8 Hz, 4-H), 6.13 (1H, d, *J* = 15.7 Hz, 2-H) and 6.79 (1H, dd, *J* = 15.7, 5.8 Hz, 3-H). ^{1}H NMR data were identical with published values.[14]

Preparation of methyl (3*R*)-3-(*p*-bromobenzoyloxy)butyrate 10

To a solution of methyl (3*R*)-3-hydroxybutyrate δ (8.2 mg) in pyridine (2 cm^3) was added *p*-bromobenzoyl chloride (6.5 mg), and the reaction mixture was left at room temperature overnight. The mixture was concentrated to dryness under reduced pressure, and the residue was purified by HPLC using MeOH–water (9 : 1) as the eluent to afford ester **10** (7.7 mg) as a pale yellow oil, $[\alpha]_D$ −21.6 (*c* 0.09 in EtOH); ν_{max} (liquid)/cm^{-1} 1725 (ester), 1611 and 1593 (C=C); *m*/*z* (EIMS) 300 (M$^+$, 21.7%), 200 (*p*-BrC_6H_4COOH, 77.6%), 183 (*p*-BrC_6H_4CO, 100.0), 100 (M$^+$ − *p*-BrC_6H_4COOH, 12.6) [*m*/*z* (HREI) Found: M$^+$, 299.9995. $C_{12}H_{13}BrO_4$ requires *M*, 299.9993]; δ_H ($CDCl_3$) 1.43 (3H, d, *J* = 6.8 Hz, 4-H_3), 2.63 (1H, dd, *J* = 13.3, 3.5 Hz, 2-H_A), 2.78 (1H, dd, *J* = 13.3, 4.5 Hz, 2-H_B), 3.65 (3H, s, $COOCH_3$), 5.50 (1H, qdd, *J* = 6.8, 4.5, 3.5 Hz, 4-H), 7.58 (2H, ArH) and 7.88 (2H, ArH); CD λ (*c* 3.77 × 10^{-4} mol dm^{-3} in EtOH)/nm 270 ($\Delta\varepsilon$ 0), 256 (+0.15), 244 (0), 230 (−0.82) and 210 (0).

p-Bromobenzoates 10 and 11 of degradation products from macrosphelide E 1

To a solution of macrosphelide E **1** (7.6 mg) in MeOH (0.2 cm^3) was added conc. H_2SO_4 (0.01 cm^3), and the reaction mixture was left at room temperature overnight. The mixture was diluted with water and extracted with diethyl ether, and the extract was evaporated under reduced pressure to give a mixture of crude **8** and **9**. To a solution of the mixture in pyridine (0.3 cm^3) was added *p*-bromobenzoyl chloride (5.3 mg), and the reaction mixture was left at room temperature overnight. The mixture was concentrated to dryness under reduced pressure,

and the residue was purified by HPLC using MeOH–water (9 : 1) as the eluent to afford *p*-bromobenzoates **10** (1.0 mg) and **11** (4.5 mg) as a pale yellow oil. The spectral data, including CD spectra, of **10** were identical with those of the synthetic compound methyl (3*R*)-3-(*p*-bromobenzoyloxy)butyrate prepared above.

***p*-Bromobenzoate 11 [methyl (4*R*,5*S*)-4,5-bis-(*p*-bromobenzoyloxy)hex-2-enoate].** *m*/*z* (EIMS) 524 (M^+, 4.7%), 324 ($M^+ - p$-BrC_6H_4COOH, 5.3%), 183 (p-BrC_6H_4CO, 100.0) [*m*/*z* (HREI) Found: M^+, 324.9462. $C_{21}H_{18}Br_2O_6$ requires *M*, 326.9464]; δ_H ($CDCl_3$) 1.50 (3H, d, J = 6.8 Hz, 6-H_3), 3.81 (3H, s, $COOCH_3$), 5.54 (1H, qd, J = 6.8, 3.2 Hz, 5-H), 5.96 (1H, ddd, J = 5.3, 3.2, 1.2 Hz, 4-H), 6.20 (1H, dd, J = 15.3, 1.2 Hz, 2-H), 7.09 (1H, dd, J = 15.3, 5.3 Hz, 3-H), 7.60 (4H, ArH) and 7.88 (4H, ArH); CD λ (c 4.08 × 10^{-4} mol dm^{-3} in EtOH)/nm 275 ($\Delta\varepsilon$ 0) and 240 (+1.26).

Formation of the (*R*)- and (*S*)-MTPA esters 13a and 13b from macrosphelide E 1

(*R*)-MTPA (2.0 mg), dicyclohexylcarbodiimide (DCC) (2.0 mg) and 4-(dimethylamino)pyridine (DMAP) (1.0 mg) were added to a CH_2Cl_2 solution (0.2 cm^3) of macrosphelide E **1** (0.6 mg), and the reaction mixture was left at room temperature for 3 h. The solvent was evaporated off under reduced pressure, and the residue was purified by HPLC using MeOH–water (4 : 1) as the eluent to afford (*R*)-MTPA ester **13a** (0.7 mg). The same reaction with **4** (0.7 mg) using (*S*)-MTPA (2.2 mg) gave ester **13b** (0.8 mg).

Ester 13a. Obtained as an amorphous powder; *m*/*z* (EI) 774 (M^+) [*m*/*z* (HREI) Found: M^+, 774.2114. $C_{36}H_{36}F_6O_{12}$ requires *M*, 774.2112]; δ_H ($CDCl_3$) 1.20 (3H, d, J = 6.6 Hz, 17-H_3), 1.25 (3H, d, J = 6.6 Hz, 19-H_3), 1.43 (3H, d, J = 6.6 Hz, 18-H_3), 2.46 (1H, dd, J = 14.9, 6.1 Hz, 2-H_A), 2.76 (1H, dd, J = 14.9, 3.0 Hz, 2-H_B), 3.45 (3H, s, OMe), 3.50 (3H, s, OMe), 5.16 (1H, qdd, J = 6.6, 6.1, 3.0 Hz, 3-H), 5.23 (1H, qd, J = 6.6, 3.2 Hz, 9-H), 5.30 (1H, qd, J = 6.6, 4.3 Hz, 15-H), 5.45 (1H, ddd, J = 7.8, 4.3, 1.2 Hz, 14-H), 5.62 (1H, ddd, J = 4.8, 3.2, 1.8 Hz, 8-H), 5.95 (1H, dd, J = 15.9, 1.2 Hz, 12-H), 5.97 (1H, dd, J = 15.9, 1.8 Hz, 6-H), 6.74 (1H, dd, J = 15.9, 7.8 Hz, 13-H), 6.91 (1H, dd, J = 15.9, 4.8 Hz, 7-H), 7.37 (6H, m, ArH) and 7.45 (4H, m, ArH).

Ester 13b. Obtained as an amorphous powder; *m*/*z* (EI) 774 (M^+) [*m*/*z* (HREI) Found: M^+, 774.2115. $C_{36}H_{36}F_6O_{12}$ requires *M*, 774.2112]; δ_H ($CDCl_3$) 1.14 (3H, d, J = 6.6 Hz, 17-H_3), 1.24 (3H, d, J = 6.6 Hz, 19-H_3), 1.49 (3H, d, J = 6.6 Hz, 18-H_3), 2.41 (1H, dd, J = 14.7, 6.1 Hz, 2-H_A), 2.77 (1H, dd, J = 14.7, 3.0 Hz, 2-H_B), 3.46 (3H, s, OMe), 3.49 (3H, s, OMe), 5.16 (1H, qdd, J = 6.6, 6.1, 3.0 Hz, 3-H), 5.18 (1H, qd, J = 6.6, 4.0 Hz, 15-H), 5.19 (1H, qd, J = 6.6, 3.3 Hz, 9-H), 5.40 (1H, ddd, J = 7.8, 4.0, 0.9 Hz, 14-H), 5.62 (1H, ddd, J = 5.0, 3.3, 1.6 Hz, 8-H), 5.98 (1H, dd, J = 15.9, 0.9 Hz, 12-H), 6.13 (1H, dd, J = 15.9, 1.6 Hz, 6-H), 6.78 (1H, dd, J = 15.9, 4.0 Hz, 13-H), 6.97 (1H, dd, J = 15.9, 3.3 Hz, 7-H), 7.37 (6H, m, ArH) and 7.45 (4H, m, ArH).

Preparation of methyl (5*R*)-5-*p*-bromobenzoyloxy)hex-2*E*-enoate 15

Methyl (5*R*)-5-(*p*-bromobenzoyloxy)hex-2*E*-enoate, $[\alpha]_D$ −16 (*c* 0.18 in EtOH),[18,19] was prepared from methyl (3*R*)-3-hydroxyhex-2*E*-enoate by a modification of the method reported previously.[19,20] To a solution of the ester (7.1 mg) in pyridine (2 cm^3) was added *p*-bromobenzoyl chloride (6 mg), and the reaction mixture was left at room temperature overnight. The mixture was concentrated to dryness under reduced pressure, and the residue was purified by HPLC using MeOH–water (9 : 1) as the eluent to afford methyl (5*R*)-5-(*p*-bromobenzoyloxyhex-2*E*-enoate **15** (5.7 mg) as a pale yellow oil, $[\alpha]_D$ −38.7 (*c* 0.15 in EtOH); ν_{max} (liquid)/cm^{-1} 1715 (ester), 1644 (C=C), 1610 and 1593 (ArC-C); *m*/*z* (EIMS) 326 (M^+, 15.2%), 226 (p-BrC_6H_4COOH, 66.8), 183 (p-BrC_6H_4CO, 100.0), 126 ($M^+ - p$-BrC_6H_4COOH, 15.3) [*m*/*z* (HREI) Found: M^+, 299.9995. $C_{14}H_{15}BrO_4$ requires *M*, 299.9993]; δ_H ($CDCl_3$) 1.39 (3H, d, J = 6.8 Hz, 6-H_3), 2.60 (2H, dd, J = 4.8, 3.7 Hz, 4-H_2), 3.73 (3H, s, $COOCH_3$), 5.28 (1H, qt, J = 6.8, 3.7 Hz, 5-H), 5.91 (1H, d, J = 15.2 Hz, 2-H), 6.95 (1H, dt, J = 15.2, 4.8 Hz, 3-H), 7.59 (2H, ArH) and 7.88 (2H, ArH); CD λ (c 4.58 × 10^{-4} mol dm^{-3} in EtOH)/nm 275 ($\Delta\varepsilon$ 0) and 239 (−1.79).

p-Bromobenzoates 10, 11 and 14 of degradation products from macrosphelide F 2

Using the same procedure as above with compound **1**, a solution of macrosphelide F **2** (3.4 mg) in MeOH (0.1 cm^3) was treated with conc. H_2SO_4 (0.02 cm^3). A solution of the resulting mixture of three crude esters in pyridine (0.2 cm^3) was treated with *p*-bromobenzoyl chloride (3.2 mg) and purified by HPLC [MeOH–water (9 : 1)] to afford *p*-bromobenzoates **10** (0.4 mg), **11** (2.1 mg) and **14** (1.8 mg) as a pale yellow oil.

***p*-Bromobenzoate 14 [methyl (5*S*)-5-(*p*-bromobenzoyloxy)-hex-2*E*-enoate].** CD λ (c 4.36 × 10^{-4} mol dm^{-3} in EtOH)/nm 275 ($\Delta\varepsilon$ 0) and 238 (+1.80). Its spectral data were identical with those of the synthetic enantiomer **15** except that the curves of the CD spectrum were the mirror image of those of **15**.

p-Bromobenzoates 10, 11 and 14 of degradation products from macrosphelide G 3

Using the same procedure as above with compound **1**, a solution of macrosphelide G **3** (4.6 mg) in MeOH (0.1 cm^3) was treated with conc. H_2SO_4 (0.02 cm^3). A solution of the resulting mixture of three esters in pyridine (0.2 cm^3) was treated with *p*-bromobenzoyl chloride (3.8 mg) and purified by HPLC [MeOH–water (9 : 1)] to afford *p*-bromobenzoates **10** (0.7 mg), **11** (2.8 mg) and **14** (2.2 mg).

Hydrogenation of macrosphelide E 1

To a solution of macrosphelide E **1** (2.2 mg) in MeOH (0.5 cm^3) was added 10% Pd/C (5.7 mg), and the reaction mixture was stirred under hydrogen atmosphere (1 atm) at room temperature for 2 h. The catalyst was filtered off and the solvent was evaporated off under reduced pressure. The residue was purified by HPLC using MeOH–water (1 : 1) as the eluent to afford compound **16** (2.0 mg) as an oil, $[\alpha]_D$ +15.0 (*c* 0.20, EtOH); λ_{max} (EtoH)/nm 215 (log ε/dm^3 mol^{-1} cm^{-1} 3.18); ν_{max} (liquid)/cm^{-1} 3448 (OH) and 1721 (ester); *m*/*z* (EI) 346 (M^+, 0.1%), 215 ($[C_{10}H_{15}O_5]^+$, 45.5), 199 ($[C_{10}H_{15}O_4]^+$, 58.4), 113 ($[C_6H_9O_2]^+$, 100) and 85 ($[C_5H_9O]^+$, 42.7) [*m*/*z* (HREI) Found: M^+, 456.1616. $C_{16}H_{26}O_8$ requires *M*, 456.1626]; δ_H ($CDCl_3$) 1.21 (3H, d, J = 6.6 Hz, 17-H_3), 1.24 (3H, d, J = 6.4 Hz, 18-H_3), 1.30 (3H, d, J = 6.6 Hz, 19-H_3), 1.60 (1H, m, 13-H_A), 1.75 (1H, m, 13-H_B), 1.77 (1H, m, 7-H_A), 2.12 (1H, m, 7-H_B), 2.34 (1H, m, 12-H_A), 2.36 (2H, m, 6-H_2), 2.52 (1H, dd, J = 14.9, 10.1 Hz, 2-H_A), 2.59 (1H, dd, J = 14.9, 2.5 Hz, 2-H_B), 2.68 (1H, m, 12-H_B), 3.68 (1H, ddd, J = 12.9, 10.6, 4.1 Hz, 8-H), 3.76 (1H, ddd, J = 10.9, 4.1, 1.9 Hz, 14-H), 4.82 (1H, qd, J = 6.63, 1.9 Hz, 15-H), 5.10 (1H, dq, J = 12.9, 6.4 Hz, 9-H) and 5.64 (1H, dqd, 10.1, 6.6, 2.5 Hz, 3-H); δ_C ($CDCl_3$) 12.31 (C-17), 16.33 (C-18), 20.48 (C-19), 25.29 (C-7), 28.47 (C-13), 30.11 (C-6), 31.44 (C-12), 42.43 (C-2), 67.09 (C-3), 69.91 (C-14), 71.87 (C-9), 73.05 (C-8), 74.59 (C-15), 169.09 (C-1), 174.34 (C-11) and 174.94 (C-5); CD λ (c 7.23 × 10^{-4} mol dm^{-3} in EtOH)/nm 238 ($\Delta\varepsilon$ 0) and 223 (+0.90).

Hydrogenation of macrosphelide I 5

Using the same procedure as above with compound **1**, a solution of macrosphelide I **5** (1.7 mg) in MeOH (0.5 cm^3) was hydrogenated in the presence of 10% Pd/C (4.7 mg), and the

resulting product was purified by HPLC [MeOH–water (1 : 1)] to afford **16** (1.5 mg), identical with the compound derived from **1**.

Formation of the (*R*)- and (*S*)-MTPA esters 17a and 17b from macrosphelide C 7

Using the same procedure as above with macrosphelide E **1**, macrosphelide C **7** (0.7 and 0.8 mg) was treated with (*R*)-MTPA (2.0 mg) and (*S*)-MTPA (2.0 mg) to afford esters **17a** (0.9 mg) and **17b** (0.7 mg), respectively.

Ester 17a. Obtained as an amorphous powder; *m*/*z* (EI) 542 (M^+) [*m*/*z* (HREI) Found: M^+, 542.1761. $C_{26}H_{29}F_3O_9$ requires *M*, 542.1763]; δ_H ($CDCl_3$) 1.30 (3H, d, *J* = 6.4 Hz, 17-H_3), 1.32 (3H, d, *J* = 6.6 Hz, 19-H_3), 1.37 (3H, d, *J* = 6.4 Hz, 18-H_3), 2.35 (1H, dddd, *J* = 13.3, 4.7, 3.4, 1.1 Hz, 8-H_A), 2.49 (1H, dd, *J* = 15.2, 8.1 Hz, 2-H_A), 2.51 (1H, dtd, *J* = 13.3, 10.1, 0.9 Hz, 8-H_B), 2.61 (1H, dd, *J* = 15.2, 3.0 Hz, 2-H_B), 3.50 (3H, s, OMe), 5.11 (1H, dqd, *J* = 10.1, 6.4, 4.7 Hz, 9-H), 5.14 (1H, dq, *J* = 7.6, 6.4 Hz, 15-H), 5.27 (1H, dqd, *J* = 8.1, 6.4, 3.0 Hz, 3-H), 5.43 (1H, ddd, *J* = 7.6, 6.3, 1.1 Hz, 14-H), 5.76 (1H, ddd, *J* = 15.9, 1.1, 0.9 Hz, 6-H), 5.86 (1H, dd, *J* = 15.9, 1.1 Hz, 12-H), 6.70 (1H, dd, *J* = 15.9, 6.3 Hz, 13-H), 6.82 (1H, ddd, *J* = 15.9, 10.1, 3.4 Hz, 7-H), 7.42 (3H, m, ArH) and 7.47 (2H, m, ArH).

Ester 17b. Obtained as an amorphous powder; *m*/*z* (EI) 542 (M^+) [*m*/*z* (HREI) Found: M^+, 542.1762. $C_{26}H_{29}F_3O_9$ requires *M*, 542.1763]; δ_H ($CDCl_3$) 1.16 (3H, d, *J* = 6.2 Hz, 17-H_3), 1.30 (3H, d, *J* = 6.6 Hz, 19-H_3), 1.37 (3H, d, *J* = 6.4 Hz, 18-H_3), 2.33 (1H, dddd, *J* = 13.3, 4.7, 3.1, 1.2 Hz, 8-H_A), 2.47 (1H, dd, *J* = 15.0, 8.2 Hz, 2-H_A), 2.51 (1H, dtd, *J* = 13.3, 10.0, 1.0 Hz, 8-H_B), 2.52 (1H, dd, *J* = 15.0, 3.0 Hz, 2-H_B), 3.52 (3H, s, OMe), 5.09 (1H, dq, *J* = 7.6, 6.2 Hz, 15-H), 5.14 (1H, dqd, *J* = 10.0, 6.4, 4.7 Hz, 9-H), 5.26 (1H, dqd, *J* = 8.2, 6.4, 3.0 Hz, 3-H), 5.41 (1H, ddd, *J* = 7.6, 6.3, 1.0 Hz, 14-H), 5.76 (1H, ddd, *J* = 15.9, 1.2, 1.0 Hz, 6-H), 6.0 (1H, dd, *J* = 15.9, 1.0 Hz, 12-H), 6.75 (1H, dd, *J* = 15.9, 6.3 Hz, 13-H), 6.81 (1H, ddd, *J* = 15.9, 10.0, 3.1 Hz, 7-H), 7.42 (3H, m, ArH) and 7.47 (2H, m, ArH).

X-Ray crystallography of macrosphelide C 7†

Macrosphelide C **7** was crystallized from hexane–CH_2Cl_2 solution by the vapor diffusion method. Crystal data: $C_{16}H_{22}O_7$, *M* = 326.34, orthorhombic, $P2_12_12_1$, *a* = 10.514(2), *b* = 28.460(2), *c* = 5.860(2) Å, *V* = 1753.4(7) Å^3, *Z* = 4, D_x = 1.236 Mg m^{-3}, *F*(000) = 696, μ(Cu-Kα) = 0.817 mm^{-1}. Data collection was performed by a Rigaku AFC5R using graphite-monochromated radiation (λ = 1.5418 Å). A total of 2473 reflections were collected until θ = 67.63 Å, in which 2185 reflections were observed [$I > 2\sigma(I)$]. The crystal structure was solved by the direct method using SHELXS-97.[22] The structure was refined by the full matrix least-squares method on F^2 using SHELXL-97.[23] In the structure refinements, non-hydrogen atoms were refined with anisotropic temperature factors. Hydrogen atoms were calculated on the geometrically ideal positions by the 'ride on' method, and were included in the calculation of structure factors with isotropic temperature factors. In the final stage, *R* = 0.0437, R_w = 0.1055 and *S* = 1.060 were obtained.

Cell-adhesion assay

This assay was carried out according to a modification of Miki's method using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT)-labeled cells.[20] HUVEC (DIA-IATRON Co., Ltd., Japan) were cultured until confluent in a 96-well plate in medium 199 (Invitrogen Corp., USA) containing 10% fetal calf serum (FCS, Invitrogen Corp.) and washed with phosphate-buffered saline (PBS, DIA-IATRON Co., Ltd.) containing 20% FCS. The HUVEC were stimulated with a solution of lipopolysaccharides (LPS, Sigma) in RPMI 1640 medium (Invitrogen Corp.) containing 10% FCS for 4 h in the presence of various concentrations of macrosphelides, and then MTT-labelled HL-60 cells were added and incubated for 40 min at 37 °C in 5% CO_2. Unbound cells were gently washed out with PBS containing 10% FCS, and DMSO was added to lyse the adherent HL-60 cells. Absorbance at 540 nm was measured using a microplate reader (Model 450, Bio-Rad).

† CCDC reference number 169025. See http://www.rsc.org/suppdata/p1/b1/b104337b/ for crystallographic files in .cif or other electronic format.

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