Isolation and Absolute Configuration Determination of Aliphatic Sulfates as the *Daphnia* Kairomones Inducing Morphological Defense of a Phytoplankton—Part 2

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4,8-Dimethylnonyl sulfate (1) and 3-methyl-4*E*-decenyl sulfate (2) were isolated from *Daphnia pulex* as the *Daphnia* kairomones that induced morphological defense of a freshwater phytoplankton *Scenedesmus gutwinskii* var. *heterospina* (NIES-802). The absolute configuration at C4 of 1 was determined by Ohrui's method applied to alcohol 3. The absolute stereochemistry at C3 of 2 was determined by ^{1}H-NMR analysis of the (*R*)-1NMA ester of alcohol 11.

Key words *Daphnia* kairomone; *Scenedesmus*; aliphatic sulfate; absolute configuration

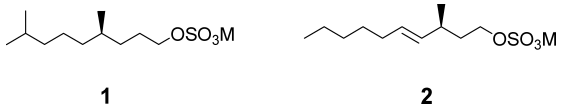
It has been believed that phytoplankton is docile and never resists its fate. Recently, it became clear that many phytoplankton resist their predators using various strategies. *Scenedesmus*, a unicellular fresh-water phytoplankton, resists its grazer by changing its morphology. Addition of filtered medium of *Daphnia*, a grazer of the plankton, to unicellular *Scenedesmus subspicatus* achieves morphological change into 2, 4, and 8 colonies within a few days. Such a change of morphology increases resistance of the colonies against grazer.[1)] This morphological change was supposed to be a self-defense mechanism acquired by the phytoplankton and triggered by a kairomone secreted from *Daphnia*. In this manner, chemical signals play important roles in the interactions among living organisms in aquatic environment. To create more accurate prey–predator interaction models and to advance the research on chemical communication in aquatic ecological system, it is essential to identify the compounds. Recently, we reported identification of the *Daphnia* kairomones that cause the morphological change in a unicellular green alga *Scenedesmus gutwinskii* var. *heterospina* (NIES-802) at 10^{-1}—10^3 ng/ml concentrations.[2,3)]

Here we report isolation and absolute configuration determination of new aliphatic sulfates **1** and **2** as the *Daphnia* kairomones. The absolute stereochemistries at C4 of **1** and C3 of **2** were determined by ^{1}H-NMR analysis of Ohrui's method[4)] applied to alcohol **3** and the (*R*)-methoxy-(1-naphthyl) acetic acid ((*R*)-1NMA) ester of alcohol **11**, respectively. Frozen *Daphnia* (10 kg; Aso Tropical Fish Co. Ltd., Osaka) was soaked with methanol (20 l×3), and the methanol solution was evaporated, the residue being treated with water (9 l). The mixture was successively extracted with hexane, dichloromethane, and butanol, and the most active butanol extract was separated by HPLC monitoring the activity to afford **1** (2.0 mg) and **2** (0.8 mg) (Fig. 1).

The molecular formula of **1** was established as $C_{11}H_{23}O_4S$ on the basis of HR-FAB-MS. The presence of a sulfate group was suggested by a fragment ion peak at *m*/*z* 97 (HSO_4^-) in the negative ion FAB-MS of **1**. The ^{1}H-NMR spectrum of **1** exhibited three doublet methyls at δ 0.93 (3H, d, J=6.1 Hz, Me-4) and 0.92 (6H, d, J=6.6 Hz, H-9 and Me-8), two protons of a methylene bearing a sulfate group at δ 4.02 (2H, t, J=6.6 Hz, H-1) and twelve methine/methylene protons at δ 1.70—1.50. Interpretation of the ^{1}H–^{1}H COSY spectrum of **1** led to a gross structure as 4,8-dimethylnonyl sulfate. To the best of our knowledge, **1** is a new compound.

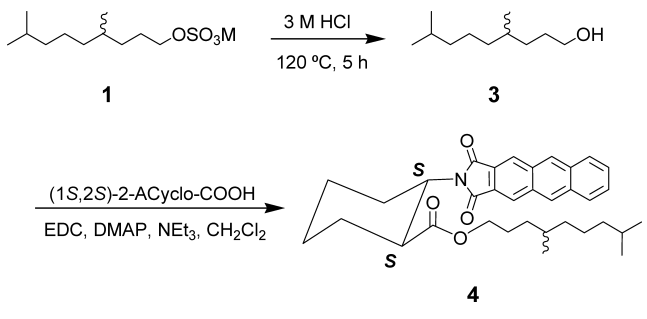
The absolute stereochemistry at C-4 of **1** was determined by the ^{1}H-NMR analysis of a (1*R*,2*R*)-2-(2,3-anthracenedicarboximido)cyclohexanecarboxylic acid ((1*R*,2*R*)-2ACyclo-COOH, Ohrui reagent[4)]) ester. The alcohol derivative **3** obtained by hydrolysis of **1** with 3 M HCl, was treated with (1*S*,2*S*)-Ohrui reagent to give the ester **4** (Chart 1). The authentic (*S*)-methyl alcohol was synthesized by the reactions shown in Chart 2. Synthesis of the authentic samples started from (*S*)-citronellal **5** which was hydrogenated over Pd–C catalyst to give the aldehyde **6**. The Wittig olefination of the aldehyde **6** with (methoxy methyl)triphenylphosphonium chloride gave the enolate intermediate, followed by hydrolysis of the intermediate to the aldehyde **7**. The aldehyde was treated with $NaBH_4$ to give 4(*S*),8-dimethylnonan-1-ol 4(*S*)-**3**. The (*S*)-alcohol was condensed with (1*R*,2*R*)- and (1*S*,2*S*)-Ohrui reagent, to yield the respective esters **8** and **9**.

The methyl proton signals of (1*S*,2*S*)-2ACyclo-COOH de-



1 **2**

Fig. 1. The Structure of Daphnia Kairomones **1** and **2**
The countercations were not identified and expressed as M.



1 **3** **4**

Chart 1. Synthesis of (1*S*,2*S*)-2-ACyclo-COOH Ester **4**

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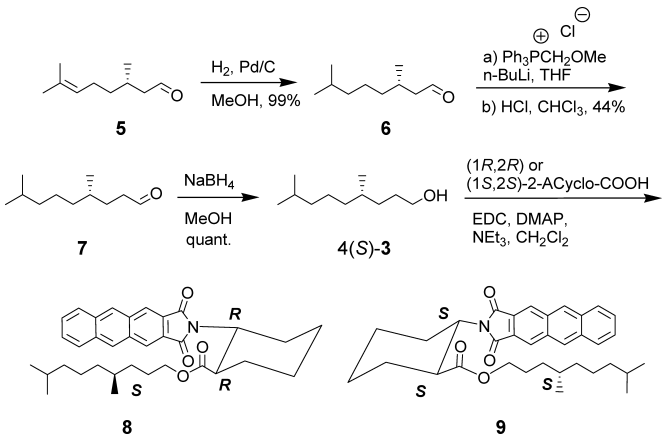

Chart 2. Synthesis of (1*R*,2*R*)-2-ACyclo-COOH Ester **8** and (1*S*,2*S*)-2-ACyclo-COOH Ester **9**

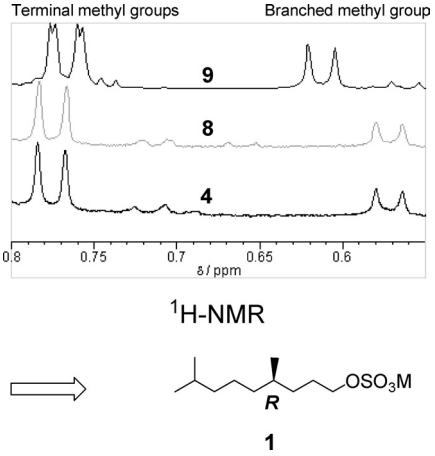

Fig. 2. Determination of the Absolute Configuration of **1**

rivative of compound **1** (**4**) appeared at δ 0.78 (6H, d, J=6.6 Hz, Me-8, H-9), 0.57 (3H, d, J=6.4 Hz, Me-4). These chemical shifts are the same with those of the methyl protons of the (1*R*,2*R*)-2ACyclo-COOH ester of 4(*S*),8-dimethylnonan-1-ol **8**, leading to the 4(*R*)-configuration of compound **1** (Fig. 2).

The molecular formula of **2** was established as $C_{11}H_{21}O_4S$ on the basis of HR-FAB-MS. The presence of a sulfate group was suggested by a fragment ion peak at *m*/*z* 97 (HSO_4^-) in the negative ion FAB-MS of **2**. The ^{1}H-NMR spectrum of **2** exhibited two olefinic protons at δ 5.48 (1H, dtd, J=15.3, 6.6, 1.0 Hz, H-5) and 5.30 (1H, ddt, J=15.3, 7.9, 1.2 Hz, H-4), two protons of a methylene bearing a sulfate group at δ 4.04 (2H, m, H-1), two methyl groups at δ 1.04 (3H, d, J=6.8 Hz, Me-3), 0.93 (3H, t, J=6.8 Hz, H-10) and eleven methine/methylene protons at δ 2.31—1.44. The ^{1}H–^{1}H COSY, HSQC and HMBC NMR spectra of **2** were consistent with the structure of 3-methyl-4-decenyl sulfate. The geometry of the double bonds in **2** was deduced to be 4*E* from the underlined coupling constants of the olefinic protons. To the best of our knowledge, **2** is a new compound.

The absolute stereochemistry at C-3 of **2** was determined by the ^{1}H-NMR analysis of (*R*)-1NMA[5,6] ester of **11**, a hydrolysis product (Chart 3). The olefin of compound **2** (0.5 mg) was hydrogenated over Pd–C catalyst, followed by hydrolysis of the sulfate **10** to an alcohol derivative **11**. The

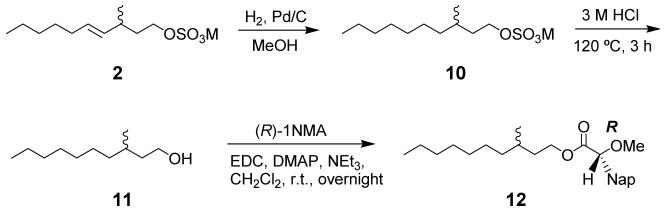

Chart 3. Synthesis of (*R*)-1NMA Ester **12**

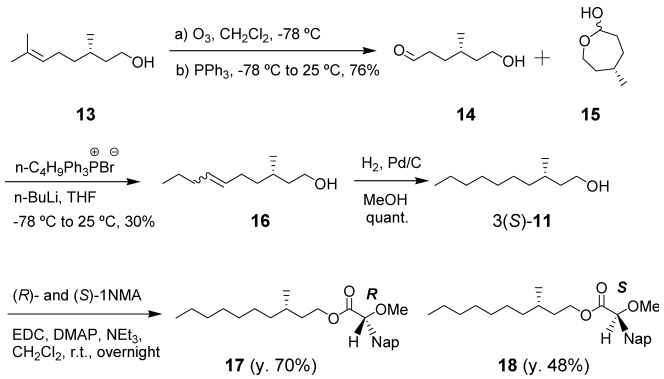

Chart 4. Synthesis of (*R*)-1NMA Ester **17** and (*S*)-1NMA Ester **18**

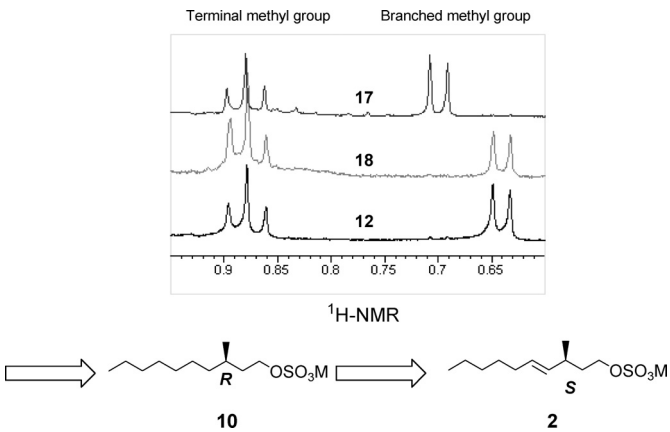

Fig. 3. Determination of the Absolute Configuration of **2**

alcohol **11** was esterified with (*R*)-1NMA to yield the (*R*)-1NMA ester **12**. The pattern of methyl protons was compared with those of the authentic samples synthesized by the reactions shown in Chart 4. (*S*)-Citronellol **13** was converted by ozonolysis to a 1/1 mixture of aldehyde **14**[7] and its hemiacetal form **15**[7] in 76% yield. The Wittig olefination of the mixture **14**+**15** with *n*-butyltriphenylphosphonium bromide gave the alcohol **16** (*Z*/*E*=1/1) in 30% yield. Reduction of the double bond under H_2 atmosphere over the Pd–C catalyst provided 3(*S*)-methyldecan-1-ol 3(*S*)-**11**. The alcohol was condensed with (*R*)- and (*S*)-1NMA, to yield the (*R*)-1NMA ester **17** and (*S*)-1NMA ester **18**.

The methyl proton signals of (*R*)-1NMA ester of **11** derived from natural **2** appeared at δ 0.88 (3H, t, J=6.8 Hz) and 0.64 (3H, d, J=6.6 Hz). These chemical shifts are the same with those of the methyl protons of the (*S*)-1NMA ester of 3(*S*)-methyldecan-1-ol **18**, leading to the 3(*S*)-configuration of compound **2** (Fig. 3).

Experimental

High-resolution MS were recorded on a JEOL JMS-SX102A spectrometer and Waters LCT Premier. ^{1}H- and ^{13}C-NMR spectra were obtained on a Bruker Avance-400 (^{1}H and ^{13}C at 400 and 100 MHz, respectively). Assignments of the proton and carbon signals were established by COSY, HSQC and HMBC spectra. Optical rotations were determined on a JASCO DIP-370 polarimeter. The structures of the compounds were established by COSY, HSQC, HMBC, and NOESY spectra. The countercation of natural sulfates was not identified and expressed as M^+.

Bioassay Each 200 ml of C medium of *S. gutwinskii* (5.0×10^2 cells/ml) is delivered into the central 30—50 wells of 96-well polystyrene tissue culture plate (CELLSTAR, Greiner Bio-one Co., Ltd.) containing the test samples (1000—0.01 ng/ml), and the outer wells are filled with distilled water to avoid dehydration of the system. The plate is covered with a plastic lid, and incubated at 20 °C (12 light/12 dark) for 10 d. A drop of the medium is placed on a Thoma's hemacytometer, and the numbers of 1-, 2-, 4-, and 8-cell types were counted under a microscope (×200).

Extraction and Isolation Frozen *Daphnia* (10 kg; Aso Tropical Fish Co., Ltd., Osaka) was soaked with methanol (20 l×3), and the methanol solution was evaporated, the residue being treated with water (9 l). The mixture was successively extracted with hexane (9 l), dichloromethane (9 l), and butanol (9 l), and the most active butanol extract (18 g) was chromatographed on a Cosmosil 75C_{18}-OPN (25 g), eluting with MeOH–H_2O in a gradient manner (1 : 1→10 : 0). The active fractions were further purified by HPLC (CAPCELLPAK C_{18} column, 5 mm, 10×250 mm, MeCN–H_2O (40 : 60) containing 250 mM $NaClO_4$ as mobile phase with the flow rate 1.0 ml/min using an RI detector) to afford **1** (2.0 mg), **2** (0.8 mg).

4(*R*),8-Dimethylnonyl Sulfate (1) ^{1}H-NMR (CD_3OD) δ: 4.02 (2H, t, J=6.6 Hz, H-1), 1.70 (2H, m, H-2), 1.58 (1H, nonet, J=6.6 Hz H-8), 1.10—1.50 (9H, m, H-3, 4, 5, 6, 7), 0.93 (3H, d, J=6.1 Hz, Me-4), 0.92 (6H, d, J=6.6 Hz, H-9 and Me-8). ^{13}C-NMR (CD_3OD) δ: 69.5 (C-1), 40.5 (C-7), 38.4 (C-5), 34.2 (C-3), 33.8 (C-4), 29.2 and 28.1 (C-2, 8), 25.9 (C-6), 23.1 and 23.0 (C-9 and Me-8), 19.7 (Me-4). HR-FAB-MS (−): *m/z* 251.1331 (Calcd for $C_{11}H_{23}O_4S$: 251.1317).

3(*S*)-Methyl-4*E*-decenyl Sulfate (2) ^{1}H-NMR (CD_3OD) δ: 5.48 (1H, dtd, J=15.3, 6.6, 1.0 Hz, H-5), 5.30 (1H, ddt, J=15.3, 7.9, 1.2 Hz, H-4), 4.04 (2H, m, H-1), 2.31 (1H, sept. J=7.1 Hz, H-3), 2.03 (2H, q. J=6.6 Hz, H-6), 1.67 (2H, m, H-2), 1.28—1.44 (6H, m, H-7, 8, 9), 1.04 (3H, d, J=6.8 Hz, Me-3), 0.93 (3H, t, J=6.8 Hz, H-10). HR-FAB-MS (−): *m/z* 249.1182 (Calcd for $C_{11}H_{21}O_4S$: 249.1161).

4(*R*),8-Dimethylnonyl (1*S*,2*S*)-2-(2,3-Anthracenedicarboximido)cyclohexanecarboxylate (4) Sulfate **1** (2.2 mg, 7.5 μmol) was dissolved in 3 M HCl (0.5 ml) and the solution was heated at 120 °C for 5 h. Dichloromethane and water were added to the solution. The organic phase was dried over Na_2SO_4, and concentrated to give the alcohol **3**. To a CH_2Cl_2 solution of the alcohol, were added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) (4.5 mg, 32 μmol), 4-dimethylaminopyridine (DMAP) (2.8 mg, 23 μmol), NEt_3 (2.6 μl, 48 μmol) and (1*S*,2*S*)-2-(2,3-anthracenedicarboximido)cyclohexanecarboxylic acid (0.4 mg, 1.0 μmol). After the mixture was stirred for 12 h at room temperature, the crude ester was purified by SiO_2 column chromatography with hexane/EtOAc solvent system to afford the ester **4** (1.4 mg). ^{1}H-NMR ($CDCl_3$) δ: 8.61 (2H, s, anthracene), 8.47 (2H, s, anthracene), 8.07 (2H, dd, J=6.6, 3.4 Hz, anthracene), 7.61 (2H, dd, J=6.6, 3.4 Hz, anthracene), 4.44 (1H, dt, J=11.7, 3.8 Hz, cyclohexane C2-H), 3.87 (2H, t, J=6.8 Hz, 6.8, –OCH_2–), 3.51 (1H, dt, J=11.7, 3.6 Hz, cyclohexane C1-H), 2.32—0.80 (12H, m, –CH–, –CH_2–), 0.78 (6H, d, J=6.6 Hz, Me-8, H-9), 0.57 (3H, d, J=6.4 Hz, Me-4).

3(*S*),7-Dimethyloctanal (6) The methanol solution of (*S*)-citronellal **5** (216 mg, 1.4 mmol) was stirred with palladium carbon (20 mg, 10%) under H_2 atmosphere. After 4 h stirring, the palladium carbon was removed by filtration, and the filtrate was concentrated *in vacuo* to give **6** (217 mg, 99%). ^{1}H-NMR ($CDCl_3$) δ: 9.74 (1H, dd, J=2.0, 2.4 Hz, H-1), 2.38 (1H, ddd, J=16.0, 5.8, 2.0 Hz, H-2), 2.21 (1H, ddd, J=16.0, 7.8, 2.4 Hz, H-2), 2.03 (1H, m, H-3), 1.52 (1H, nonet, J=6.6 Hz, H-7), 1.10—1.38 (6H, m, H-4, 5, 6), 0.95 (3H, d, J=6.8 Hz, Me-3), 0.86 (6H, d, J=6.6 Hz, H-8 and Me-7). ^{13}C-NMR ($CDCl_3$) δ: 202.4, 50.9, 38.9, 37.0, 28.0, 27.8, 24.6, 22.5, 22.4, 19.8.

4(*S*),8-Dimethylnonanal (7) To a suspension of (methoxymethyl)triphenylphosphonium chloride (960 mg, 2.8 mmol) in THF (4 ml), cooled at 0 °C, was added 1.6 M solution of *n*-BuLi in hexane (1.75 ml, 2.8 mmol). The red solution was stirred 30 min at 0 °C and **6** (217 mg, 1.4 mmol) was added. The reaction mixture was stirred for 12 h at room temperature and then quenched with 1 M HCl, extracted with diethyl ether (×3), dried over anhydrous magnesium sulfate and concentrated partially under reduced pressure. The residue was filtered to remove the solid triphenylphosphine oxide and the filtrate was concentrated under reduced pressure. The enol ester obtained was then dissolved in 2 ml of chloroform and 0.15 ml of 12 M HCl was added. The solution was stirred for 4 h at room temperature and the chloroform was evaporated. Diethyl ester and water were added and the aqueous phase was extracted with diethyl ester (×2) and the combined organic layers were dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was purified by flash chromatography eluting with ethyl acetate : hexane (5 : 95) to yield 101 mg of the desired aldehyde **7** (0.59 mmol, 42%). ^{1}H-NMR ($CDCl_3$) δ: 9.76 (1H, t, J=1.8 Hz, H-1), 2.41 (2H, m, H-2), 1.64 (1H, m, H-4), 1.51 (1H, m, H-8), 1.08—1.45 (8H, m, H-3, 5, 6, 7), 0.87 (3H, d, J=5.4 Hz, Me-4), 0.86 (6H, d, J=6.6 Hz, H-9 and Me-8). $[\alpha]_D^{25}$ −1.8° (c=2.1, $CHCl_3$).

4(*S*),8-Dimethylnonan-1-ol (4(*S*)-3) To a methanol solution of the aldehyde **7** (65 mg, 0.38 mmol), sodium borohydride (29 mg, 0.77 mmol) was added. The reaction mixture was stirred for 3 h at room temperature, poured into water (20 ml), extracted with diethyl ether (20 ml×3). The organic layers were combined, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to provide the desired alcohol 4(*S*)-**3** (65 mg, 0.38 mmol). ^{1}H-NMR ($CDCl_3$) δ: 3.62 (2H, t, J=6.8 Hz, H-1), 1.60 (2H, m, H-2), 1.51 (1H, m, H-6), 1.07—1.42 (9H, m, H-3, 4, 5, 6, 7), 0.86 (3H, d, J=6.3 Hz, Me-4), 0.86 (6H, d, J=6.6 Hz, H-9 and Me-8). ^{13}C-NMR ($CDCl_3$) δ: 63.5 (C-1), 39.4 (C-7), 37.3 (C-5), 33.0 (C-3), 32.7 (C-4), 30.4 (C-2), 28.0 (C-8), 24.8 (C-6), 22.8 and 22.7 (C-9 and Me-8), 19.7 (Me-4). $[\alpha]_D^{25}$ −1.3° (c=3.2, $CHCl_3$).

4(*S*),8-Dimethylnonyl (1*R*,2*R*)-2-(2,3-Anthracenedicarboximido)cyclohexanecarboxylate (8) To a CH_2Cl_2 solution of the alcohol 4(*S*)-**3** (1 mg, 5.8 μmol), were added EDC (3.3 mg, 17 μmol), DMAP (2.2 mg, 18 μmol), NEt_3 (1.9 μl, 26 μmol) and (1*R*,2*R*)-2-(2,3-anthracenedicarboximido)cyclohexanecarboxylic acid (0.4 mg, 1.0 μmol). After the mixture was stirred for 12 h at room temperature, the crude ester was purified by SiO_2 column chromatography with hexane/EtOAc solvent system to afford the ester **8** (0.5 mg, 0.9 μmol). ^{1}H-NMR ($CDCl_3$) δ: 8.61 (2H, s, anthracene), 8.47 (2H, s, anthracene), 8.07 (2H, dd, J=6.6, 3.4 Hz, anthracene), 7.61 (2H, dd, J=6.6, 3.4 Hz, anthracene), 4.44 (1H, dt, J=11.7, 3.8 Hz, cyclohexane C2-H), 3.87 (2H, t, J=6.8 Hz, –OCH_2–), 3.51 (1H, dt, J=11.7, 3.6 Hz, cyclohexane C1-H), 2.32—0.80 (12H, m, –CH–, –CH_2–), 0.78 (6H, d, J=6.6 Hz, Me-8, H-9), 0.57 (3H, d, J=6.4 Hz, Me-4).

4(*S*),8-Dimethylnonyl (1*S*,2*S*)-2-(2,3-Anthracenedicarboximido)cyclohexanecarboxylate (9) To a CH_2Cl_2 solution of the alcohol 4(*S*)-**3** (1 mg, 5.8 μmol), were added EDC (6.7 mg, 35 μmol), DMAP (4.2 mg, 34 μmol), NEt_3 (3.8 μl, 52 μmol) and (1*S*,2*S*)-2-(2,3-anthracenedicarboximido)cyclohexanecarboxylic acid (1.2 mg, 3.2 μmol). After the mixture was stirred for 24 h at room temperature, the crude ester was purified by SiO_2 column chromatography with hexane/EtOAc solvent system to afford the ester **9** (0.9 mg, 1.7 μmol). ^{1}H-NMR ($CDCl_3$) δ: 8.61 (2H, s, anthracene), 8.47 (2H, s, anthracene), 8.07 (2H, dd, J=6.6, 3.2 Hz, anthracene), 7.61 (2H, dd, J=6.6, 3.2 Hz, anthracene), 4.44 (1H, dt, J=11.7, 3.9 Hz, cyclohexane C2-H), 3.88 (2H, m, –OCH_2–), 3.51 (1H, dt, J=11.7, 3.4 Hz, cyclohexane C1-H), 2.31—0.80 (12H, m, –CH–, –CH_2–), 0.77 (6H, dd, J=6.6, 1.5 Hz, Me-8, H-9), 0.61 (3H, d, J=6.6 Hz, Me-4).

3(*R*)-Methyldecyl (*R*)-Methoxy-(1-naphthyl)acetate (12) The methanol solution of **2** (0.5 mg, 1.8 μmol) was stirred with palladium carbon (10%) under H_2 atmosphere. After 2 h stirring, the palladium carbon was removed by filtration, and the filtrate was concentrated *in vacuo* to give saturated sulfate **10** (0.5 mg, quant.). The sulfate was dissolved in 3 M HCl (0.5 ml) and the solution was heated at 120 °C for 3 h. Dichloromethane (CH_2Cl_2) was added to the solution. The organic phase was dried over Na_2SO_4 and concentrated to give the alcohol **11**. To a CH_2Cl_2 solution (50 μl) of the alcohol **11** were added EDC (1.7 mg, 9 μmol), DMAP (1.1 mg, 9 μmol), NEt_3 (1.8 μl, 12 μmol) and (*R*)-methoxy-(1-naphthyl)acetic acid (1NMA) (1.0 mg, 5 μmol). The mixture was allowed to stand at room temperature for 48 h, and the crude ester was purified by SiO_2 column chromatography with hexane : EtOAc (9 : 1) solvent system to afford the (*R*)-1NMA ester **12** (0.3 mg, 1.3 μmol, 70%). ^{1}H-NMR ($CDCl_3$) δ: 8.27 (1H, d, J=8.0 Hz), 7.84 (1H, t, J=9 Hz), 7.60 (1H, d, J=7.1 Hz), 7.49 (3H, m), 5.36 (1H, s), 4.12 (2H, m), 3.45 (3H, s), 1.12—1.60 (15H, m), 0.88 (3H, t, J=6.8 Hz), 0.64 (3H, d, J=6.6 Hz). HR-EI-MS: *m/z* 370.2491 (Calcd for $C_{24}H_{34}O_3$: 370.2508).

6-Hydroxy-4-methylhexanal (14) (*S*)-Citronellol **13** (272 mg, 1.7 mmol) in CH_2Cl_2 (15 ml) was treated with ozone at −78 °C. Then 913 mg (3.5 mmol) of triphenylphosphine was added to the solution. After stirring for 4 h at room temperature, H_2O was added, and the mixture was extracted with ether. The organic layer was dried over Na_2SO_4, concentrated *in vacuo* and purified by flash chromatography on silica gel, which gave a

mixture (2/1) of **14** and its hemiacetal form **15** (191 mg, 76%). **14**: ^{1}H-NMR ($CDCl_3$) δ: 9.76 (1H, t, J=1.7 Hz), 3.62—3.73 (2H, m), 2.47 (1H, dddd, J=1.7, 6.3, 8.5, 17.3 Hz), 2.43 (1H, dddd, J=1.7, 6.7, 8.4, 17.3 Hz), 1.35—1.74 (5H, m), 0.89 (3H, d, J=6.2 Hz). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 202.6, 60.7, 41.6, 39.4, 29.1, 28.8, 19.4; hemiacetal **15**: ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.14 (1H, dd, J=5.3, 9.2 Hz), 3.90 (1H, bt, J=12.8 Hz), 3.53 (1H, dt, J=12.8, 3.4 Hz), 1.30—1.74 (7H, m), 0.91 (3H, d, J=6.2 Hz). ^{13}C-NMR ($CDCl_3$) δ: 96.5, 60.2, 39.2, 36.3, 34.0, 30.9, 23.2.

3(*S*)-Methyl-6-hexcen-1-ol (16) 1.6 M solution of *n*-BuLi in hexane (4.9 ml, 7.8 mmol) was added dropwise to a suspension of *n*-butyltriphenylphosphonium bromide (2.5 g, 7.8 mmol) in THF (10 ml). The mixture was stirred at −30 °C for 20 min, cooled at −78 °C and a solution of **14** and **15** (224 mg, 1.6 mmol) in THF (3 ml) was added. The mixture was stirred at the same temperature for 2 h, poured into water, and extracted with diethyl ether. The organic layer was dried over Na_2SO_4. After concentration, the crude product was purified by flash chromatography to give the alcohol **16** (Z/E=1/1) (80 mg, 30% yield). ^{1}H-NMR ($CDCl_3$) δ: 5.28—5.38 (4H, m, H-6, 7), 3.63 (4H, m, H-1), 1.98 (8H, m, H-5, 8), 1.56 (2H, m, H-3), 1.10—1.40 (12H, m, H-2, 4, 9), 0.87 (6H, t, J=6.6 Hz, H-10), 0.86 (6H, d, J=6.8 Hz, Me-3). ^{13}C-NMR ($CDCl_3$) δ: 130.2, 130.0, 129.8 and 129.5 (C-6, 7), 60.9 (C-1), 39.8 (C-2), 37.1, 37.0 (C-4), 34.7 (*trans* C-8), 30.0, 29.3, 29.2 and 29.0 (*trans* C-5 and 3, *cis* C-8 and 3), 24.7 (*cis* C-5), 22.9 and 22.7 (C-9), 19.5 (Me-3), 13.8 and 13.6 (C-10).

3(*S*)-Methyldecan-1-ol (3(*S*)-11) The methanol solution of **16** (58 mg, 0.34 mmol) was stirred with palladium carbon (6 mg, 10%) under H_2 atmosphere. After 3 h stirring, the palladium carbon was removed by filtration, and the filtrate was concentrated *in vacuo* to give 3(*S*)-**11** (60 mg, quant.). ^{1}H-NMR ($CDCl_3$) δ: 3.59 (2H, m, H-1), 1.50 (1H, m, H-3), 1.05—1.35 (14H, overlap, H-2, 4, 5, 6, 7, 8, 9), 0.79—0.84 (6H, overlap, C-9 and Me-3). ^{13}C-NMR ($CDCl_3$) δ: 60.8, 39.7, 37.1, 31.8, 29.8, 29.5, 29.3, 26.9, 22.6, 19.5, 14.0. $[\alpha]_D^{25}$ −2.7° (c=2.2, $CHCl_3$).

3(*S*)-Methyldecyl (*R*)-Methoxy-(1-naphthyl)acetate (17) To a CH_2Cl_2 solution (50 μl) of 3(*S*)-**11** (1 mg, 5.8 μmol) were added EDC (6.7 mg, 35 μmol), DMAP (4.3 mg, 35 μmol), NEt_3 (7.3 μl, 52 μmol) and (*S*)-methoxy-(1-naphthyl)acetic acid (1NMA) (3.8 mg, 17 μmol). After the mixture was allowed to stand for 12 h at room temperature, the crude ester was purified by SiO_2 column chromatography with CH_2Cl_2 solvent system to afford the (*S*)-1NMA ester **17** (1.5 mg, 4.1 mmol, 70%). ^{1}H-NMR ($CDCl_3$) δ: 8.28 (1H, d, J=8.2 Hz), 7.84 (1H, t, J=8.3 Hz), 7.60 (1H, d, J=7.1 Hz), 7.49 (3H, m), 5.36 (1H, s), 4.12 (2H, t, J=6.6 Hz), 3.45 (3H, s), 1.12—1.60 (15H, m), 0.88 (3H, t, J=6.8 Hz), 0.70 (3H, d, J=6.6 Hz). HR-EI-MS: m/z 370.2491 (Calcd for $C_{24}H_{34}O_3$: 370.2508).

3(*S*)-Methyldecyl (*S*)-Methoxy-(1-naphthyl)acetate (18) To a CH_2Cl_2 solution (50 μl) of 3(*S*)-**11** (1 mg, 5.8 μmol) were added EDC (6.7 mg, 35 μmol), DMAP (4.3 mg, 35 μmol), NEt_3 (7.3 μl, 52 μmol) and (*S*)-methoxy-(1-naphthyl)acetic acid (1NMA) (3.8 mg, 17 μmol). After the mixture was allowed to stand for 12 h at room temperature, the crude ester was purified by SiO_2 column chromatography with CH_2Cl_2 solvent system to afford the (*S*)-1NMA ester **18** (0.6 mg, 2.8 μmol, 48%). ^{1}H-NMR ($CDCl_3$) δ: 8.27 (1H, d, J=8.0 Hz), 7.84 (1H, t, J=8.9 Hz), 7.60 (1H, d, J=7.1 Hz), 7.49 (3H, m), 5.36 (1H, s), 4.12 (2H, m), 3.45 (3H, s), 1.12—1.60 (15H, m), 0.88 (3H, t, J=6.8 Hz), 0.64 (3H, d, J=6.6 Hz). HR-EI-MS: m/z 370.2491 (Calcd for $C_{24}H_{34}O_3$: 370.2508).

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