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Tumor Chemopreventive Activity of 3-*O*-Acylated (−)-epigallocatechins

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Abstract—In order to seek promising cancer chemopreventive agents, we assessed the antitumor promoting activities of 3-*O*-octanoyl- or 3-*O*-(2-methyloctanoyl)-(−)-epigallocatechins, inhibiting markedly the activation of Epstein–Barr virus early antigen, in a two-stage mouse skin carcinogenesis assay. As a result, these derivatives inhibited a papilloma formation 1.3–1.6-fold more strongly than (−)-epigallocatechin gallate well established as anti-tumor promoter.

Introduction

Green tea catechins have preventive activities against cancer,[1,2] atherosclerosis,[3,4] diabetes,[5] cold syndrome,[6] and so on. Recently, it was proposed that the catechins contribute to cancer prevention by multiple pathways involving antioxidative[7] and antiangiogenic[8] actions as well as ulokinase-[9] and telomerase-inhibiting activities.[10] In order to produce the satisfactory effects for human diseases, however, the structures of catechins need to be modified because of their low plasma and tissue concentrations in body.[11] We therefore tried to synthesize 3-*O*-acylated (−)-epigallocatechins (EGCs) in order to improve their pharmacokinetic profile such as cell membrane- and tissue-permeability. Straight-chain acids of C_4–C_{18} were introduced after acid chloride formation at the C-3 hydroxy group of (−)-EGC (**1**), being less responsible for radical scavenging action[12,13] than the phenolic hydroxyl groups, yielding 3-*O*-acyl-(−)-EGCs (**3a**–**3h**),[14] respectively. Furthermore, branched-chain acyl bearing derivatives: 3-*O*-[(*RS*)-2-methyloctanoyl]-(**4**) and 3-*O*-[(*RS*)-2-methyldecanoyl]-(−)-EGC (**5**) were prepared by the same method in expectation that the 2-methyl group could shield the ester bond at the C-3 acyloxy moiety from esterase-mediated cleavage in body, leading to maintenance of a high tissue concentration. These synthetic 3-*O*-acyl-(−)-EGCs were assessed for the inhibitory effects on the Epstein–Barr virus early antigen (EBV-EA) activation in Raji cells induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in order to seek promising antitumor promoting candidates for cancer chemoprevention. As a result, the (−)-EGCs possessing an acyl group of carbon number (C_8–C_{11}) showed the strong inhibitions.[14] In the present work, we therefore tried to examine the antitumor promoting activities of the promising compounds in a two-stage mouse skin carcinogenesis test (Fig. 1).

Results and Discussion

The EBV-EA activation assay of the synthetic 3-*O*-acyl-(−)-EGCs indicated that the following derivatives with a fatty acid (C_8–C_{11}) have ca. 1.7–3-fold higher inhibitory

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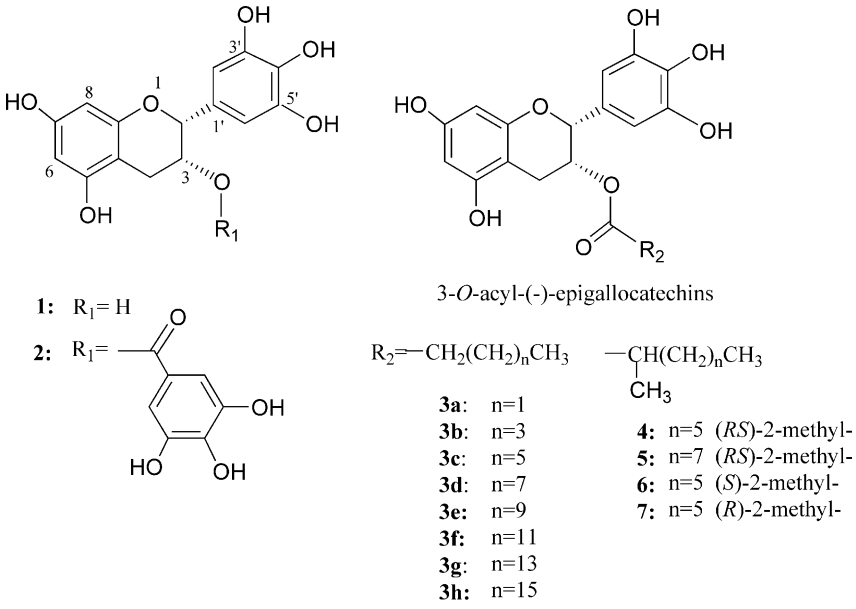


Figure 1. Structures of catechins and 3-*O*-acyl-(−)-epigallocatechins.

effects than (−)-EGCG (**2**) at 1×10^3 mol ratios/TPA: 3-*O*-octanoyl-(−)-EGC (**3c**), 3-*O*-decanoyl-(−)-EGC (**3d**), 3-*O*-[(*RS*)-2-methyloctanoyl]-(−)-EGC (**4**) and 3-*O*-[(*RS*)-2-methyldecanoyl]-(−)-EGC (**5**).[14] Since the 3-*O*-octanoyl- and 3-*O*-decanoyl-derivatives showed almost the same efficacies, we focused on the octanoyl-series such as **3c** and **4** to compare with (−)-EGC (**1**) and (−)-EGCG (**2**) for the antitumor promoting activities in a two-stage mouse skin carcinogenesis test. Furthermore, since **4** was found to consist of (*S*)-2-methyl- and (*R*)-2-methyl-diastereomers in a 1:1 ratio in its ^{1}H NMR spectrum (see Experimental), 3-*O*-[(*S*)-2-methyloctanoyl]-(−)-EGC (**6**) and 3-*O*-[(*R*)-2-methyloctanoyl]-(−)-EGC (**7**) were separately prepared and subjected to this test for the purpose of examining whether or not there exists discrepancy between the diastereomers in the inhibitory activity. Thus, (*S*)-(**12**) and (*R*)-2-methyloctanoic acid (**13**) were synthesized using chiral auxiliary 4-benzyl-2-oxazolidinones[15,16] through sequential diastereoselective α-methylation of (4*S*)- (**8**) and (4*R*)-3-(octanoyl)-4-benzyl-2-oxazolidinone (**9**) to [3(2′*S*),4*S*]- (**10**) and [3(2′*R*),4*R*]-3-(2-methyloctanoyl)-isomer (**11**), chromatographic purification and LiOOH-catalyzed hydrolysis, respectively. Acids **12** and **13** indicated the specific optical rotations of mutually opposite figures ($[\alpha]_D$ +16.2°; −15.3°),[17] respectively. They were in turn converted, after acid chloride formation, to 3-*O*-[(*S*)-2-methyloctanoyl]-(−)-EGC (**6**) and 3-*O*-[(*R*)-2-methyloctanoyl]-(−)-EGC (**7**), respectively, in the conventional way (see Experimental). In the 400 MHz ^{1}H NMR spectra, neither of **6** and **7** showed any signals due to the respective 2-methyl-isomers **7** and **6** (Fig. 2).

The two-stage mouse skin carcinogenesis test was performed with 7,12-dimethylbenz[*a*]anthracene (DMBA) as an initiator and TPA as a tumor promoter according to the reported procedure.[18]

Figures 3(a) and 4(a) showed the time-course percentage of mice bearing papillomas which were treated with an acetone solution of the 3-*O*-acylated (−)-EGC, (−)-EGC (**1**), (−)-EGCG (**2**) or acetone only (control). Papillomas were observed on 100% mice of the control group in 9 weeks after TPA treatment. On the other hand, papillomas were detected on 20, 10 and 30% mice treated with (−)-EGC (**1**), (−)-EGCG (**2**) and 3-*O*-octanoyl-(−)-EGC (**3c**), respectively, whereas they were not observed on the mice operated with 3-*O*-[(*RS*)-2-methyloctanoyl]-(−)-EGC (**4**) in 9 weeks. As shown in Figure 3(b), (−)-EGC (**1**) and (−)-EGCG (**2**) achieved

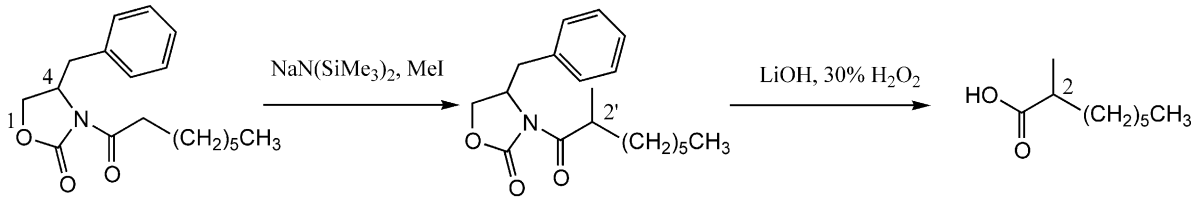


Figure 2. Synthesis of (*S*)-2- (**12**) and (*R*)-2-methyloctanoic acid (**13**) using chiral auxililary.

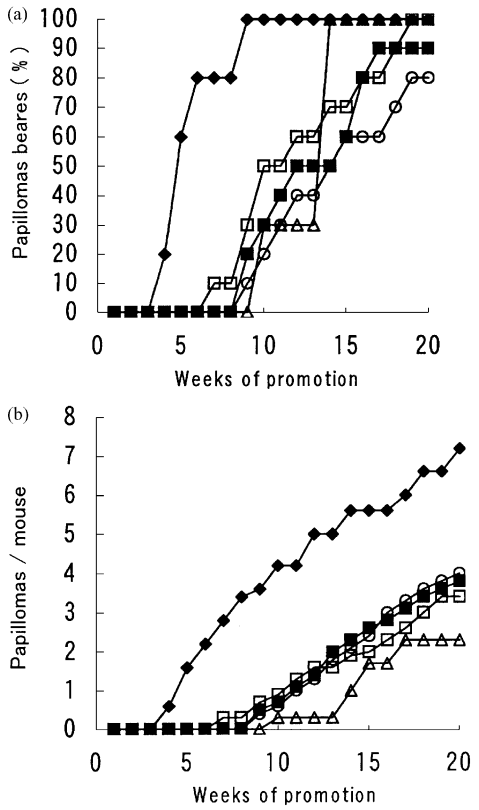


Figure 3. Antitumor promoting activities of **1**, **2**, **3c** and **4** in a two-stage mouse skin carcinogenesis test: (a) percentage of mice bearing papillomas (b) average number of papillomas per mouse [◆, positive control, TPA alone; ■, TPA + **1** ($p<0.05$); ○, TPA + **2** ($p<0.05$); □, TPA + **3c** ($p<0.05$); △, TPA + **4** ($p<0.005$)].

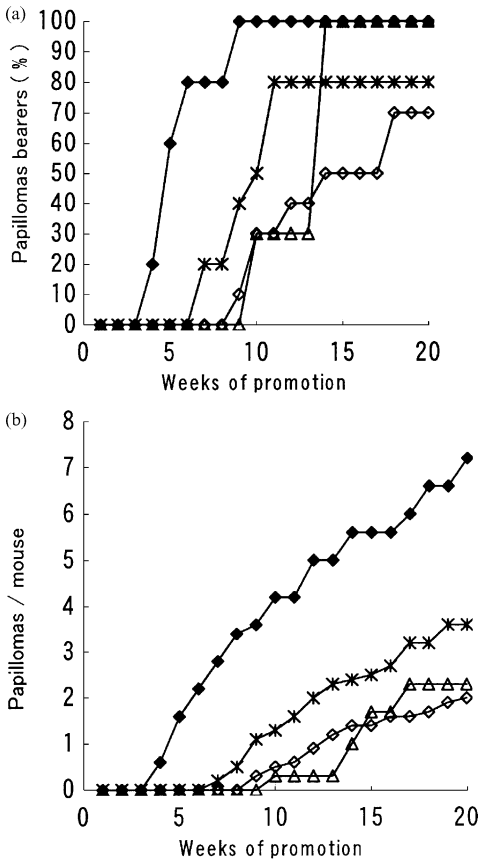
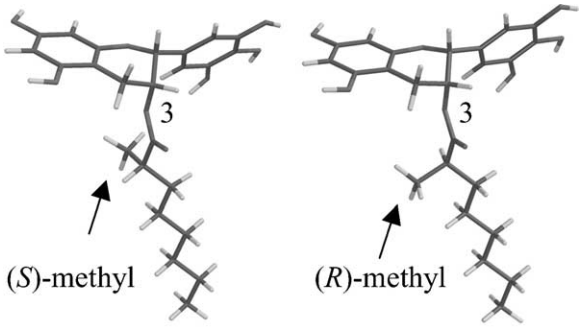


Figure 4. Antitumor promoting activities of **4**, **6** and **7** in a two-stage mouse skin carcinogenesis test: (a) percentage of mice bearing papillomas (b) average number of papillomas per mouse [◆, positive control, TPA alone; △, TPA + **4** ($p<0.005$); ◇, TPA + **6** ($p<0.005$); ∗, TPA + **7** ($p<0.05$)].

almost the same efficacies. Moreover, the average papilloma number per mouse treated with **1**, **2** and **4**, were, 3.8, 4.0 and 2.3, respectively, to 7.2 of control mouse in 20 weeks, indicating that they inhibited the papilloma formation by 47, 44 and 68%, respectively. Thus, **4** was ca. 1.5-fold more effective than **1** and **2**. 3-*O*-octanoyl-(−)-EGC (**3c**) showing 53% inhibition seems a little more active than **1** and **2** though it was inferior to **4**. It is therefore most likely that the inhibitory effect of papilloma fomation is enhanced by introducing an acyl group to (−)-EGC (**1**). Interestingly, there existed a difference in the inhibition rate of papilloma formation between the two diastereomers **6** and **7** in 20 weeks, that is the inhibition rate (72%) of 3-*O*-[(*S*)-2-methyloctanoyl]-(−)-EGC (**6**) was higher than the rate (50%) of the (*R*)-isomer **7** [Fig. 4(b)]. The reason for such a difference in the inhibition remains elusive. The most stable conformations of **6** and **7** were searched using the molecular mechanics method with MMFF94 force field[19] (Fig. 5).

It was indicated that the (2*S*)-methyl group is displaced on the O–CO–C plane of the C-3 acyloxy group in **6**, whereas the (2*R*)-methyl group is oriented perpendicularly to the O–CO–C plane in **7**. We speculated that the differential spatial proximity of the 2-methyl group and the ester bond moiety between **6** and **7** might be associated with their resistivity toward catalytic cleavage by an esterase in the dermal tissue of mice.



3-*O*-[(*S*)-2-methyloctanoyl]-(−) -EGC (**6**)

3-*O*-[(*R*)-2-methyloctanoyl]-(−)-EGC (**7**)

Figure 5. The global minimum-energy conformations of 3-*O*-[(*S*)-2-methylocatanoyl]-(−)-EGC (**6**) and 3-*O*-[(*R*)-2-methyloctanoyl]-(−)-EGC (**7**). They were generated using the molecular mechanics method with MMFF94 force field by repeating rotations (360° at 60° intervals) of rotatable ester C–C and C–O bonds in the side chain followed by optimization.

Conclusion

The introduction of the octanoyl group or 2-methyloctanoyl group at C-3 of (−)-EGC (**1**) enhanced the antitumor promoting activity of **1** on DMBA-TPA two-stage mouse skin carcinogenesis test. As exemplified,

(2*S*)-isomer **6** was 1.6-fold more active than (−)-EGCG (**2**) well established as an antitumor promoter. Thus, 3-*O*-acyl-(−)-EGCs such as **3c**, **4** and **6** could be good candidates for cancer chemoprevention.

Experimental

Melting points were determined on a Yanagimoto MP-32 micromelting point apparatus and are uncorrected. IR spectra were recorded on Shimadzu FTIR-8400 infrared spectrophotometer. Low-resolution (LR)-FABMS spectra measured on a JEOL JMS-HX 100 instrument, whereas high resolution (HR)- and LR-electron impact (EI) MS spectra, on JEOL The Tandem MStation JMS-700. ^{1}H NMR spectra were recorded on JEOL EX-270 (270 MHz), Bruker AX-300 (300 MHz), JEOL EX-400 (400 MHz) and JEOL JNM-GX 500 (500 MHz) instruments using tetramethylsilane (TMS) as an internal standard. Multiplicities were abbreviated as s = singlet, d = doublet, t = triplet, q = quartet, st = sextet, m = multiplet, dd = doublet of doublet, brs = broad singlet, brd = broad doublet. Analytical TLC and preparative TLC were performed using Silica gel 60 F_{254} (Merck, 0.25 mm) and Silica gel 60 F_{254} (Merck, 1 mm) glass plates, respectively. Preparative HPLC was performed with LC-908 (Japan Analytical Industry, Co. Ltd.) using a GS-320 column (21.5 mm ID×500 mm) and MeOH as an eluent. All extracted solvents were dried over Na_2SO_4, followed by evaporation in vacuo. Specific-pathogen-free (SPF) 6-week-old female ICR mice were purchased from Japan SLC Inc. (Shizuoka, Japan).

Two-stage carcinogenesis test on mouse skin

The ICR mice were housed in a SPF mouse room at five per polycarbonate cage and were given food and water at all the times throughout the experiment. Animals were divided into seven experimental groups 10 mice each. The back of each mouse was shaved with surgical clippers one day before starting the test, and the mice were topically treated with 100 μg of DMBA (0.1 mL of a 390 nmol solution in acetone) as an initiating treatment. One week after the initiation, papilloma formation was promoted by application twice a week of 1 μg of TPA (0.1 mL of a 1.7 nmol solution in acetone) to the skin. One h before each TPA treatment, the mice were treated with 0.1 mL of the test compounds (85 nmol in acetone). The incidence and numbers of papillomas were observed weekly over the course of 20 weeks.

General procedure for the synthesis of (−)-EGCs possessing a straight-chain acyl group

(−)-EGC (3.27 mmol), acid chloride (3.60 mmol) and trifluoroacetic acid (6.54 mmol) were dissolved in tetrahydrofuran (10 mL), and the solution was stirred for 24 h under an Ar gas. After concentration of the reaction mixture, the residue was extracted with AcOEt, which was washed with satd NaCl. The organic layer was concentrated in vacuo to give a residue, which was purified by preparative TLC with $CHCl_3$/MeOH (13:1) two developments) as an eluent, followed by freeze-drying to afford a white powder.

3-*O*-Butyryl-(−)-EGC (3a).[20] 46.4% yield. $[\alpha]_D^{19}$ −97.1° (EtOH, *c* 0.58); IR ν_{max} (KBr) 3401, 2964, 1718, 1701, 1637, 1625, 1541, 1523, 1509, 1460, 1340, 1259, 1186, 1148, 1096, 1017, 825, 737 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD) δ: 0.76 (3H, t, *J* = 7.3 Hz, –$COCH_2CH_2C\underline{H}_3$)), 1.47 (2H, st, *J* = 7.4 Hz, –$COCH_2C\underline{H}_2CH_3$), 2.18 (2H, t, *J* = 7.3 Hz, –$COC\underline{H}_2CH_2CH_3$), 2.76 (1H, AB of ABX, *J* = 2.3 and 17.5 Hz, H-4), 2.91 (1H, AB of ABX, *J* = 4.5 and 17.5 Hz, H-4), 4.82 (1H, s, H-2), 5.35 (1H, m, H-3), 5.91 (1H, d, *J* = 2.3 Hz, H-6 or H-8), 5.94 (1H, d, *J* = 2.3 Hz, H-8 or H-6), 6.47 (2H, s, H-2′ and H-6′); FABMS: *m*/*z* 377.13 $[M+H]^+$; FABHRMS *m*/*z*: 377.1261 ($[M+H]^+$, calcd for $C_{19}H_{21}O_8$: 377.1236).

3-*O*-Hexanoyl-(−)-EGC (3b). 37.9% yield. $[\alpha]_D^{20}$ −47.3° (EtOH, *c* 1.27); IR ν_{max} (KBr) 3398, 2932, 1706, 1636, 1466, 1340, 1252, 1182, 1146, 1098, 1016, 824, 735 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD) δ: 0.82 (3H, t, *J* = 7.1 Hz, –$COCH_2CH_2CH_2CH_2C\underline{H}_3$), 1.11 (2H, m, –$COCH_2CH_2CH_2CH_2C\underline{H}_2CH_3$), 1.19 (2H, m, –$COCH_2CH_2C\underline{H}_2CH_2CH_3$), 1.43 (2H, m, –$COCH_2C\underline{H}_2CH_2CH_2CH_3$), 2.19 (2H, t, *J* = 7.1 Hz, –$COC\underline{H}_2CH_2CH_2CH_2CH_3$), 2.77 (1H, AB of ABX, *J* = 2.2 and 17.2 Hz, H-4), 2.90 (1H, AB of ABX, *J* = 4.3 and 17.2 Hz, H-4), 5.33 (1H, m, H-3), 5.90 (1H, d, *J* = 2.3 Hz, H-6 or H-8), 5.94 (1H, d, *J* = 2.3 Hz, H-8 or H-6), 6.46 (2H, s, H-2′ and H-6′); EIMS: *m*/*z* 404.2 $[M]^+$; EIHRMS *m*/*z*: 404.1493 ($[M]^+$, calcd for $C_{21}H_{24}O_8$: 404.1471).

3-*O*-Octanoyl-(−)-EGC (3c). 43.7% yield. $[\alpha]_D^{19}$ −96.6° (EtOH, *c* 0.45); IR ν_{max} (KBr) 3399, 2928, 1718, 1702, 1629, 1618, 1541, 1522, 1458, 1378, 1344, 1256, 1187, 1145, 1099, 1016, 828, 733 cm^{-1}; ^{1}H NMR (270 MHz, CD_3OD) δ: 0.66 (3H, t, *J* = 7.3 Hz, –$COCH_2CH_2(CH_2)_4C\underline{H}_3$), ca. 0.98 (8H, m, –$COCH_2CH_2(C\underline{H}_2)_4CH_3$), 1.24 (2H, st, *J* = 7.3 Hz, –$COCH_2C\underline{H}_2(CH_2)_4CH_3$), 2.02 (2H, t, *J* = 7.3 Hz,–$COC\underline{H}_2CH_2(CH_2)_4CH_3$), 2.57 (1H, AB of ABX, *J* = 2.2 and 17.3 Hz, H-4), 2.70 (1H, AB of ABX, *J* = 4.6 and 17.3 Hz, H-4), 4.67 (1H, s, H-2), 5.13 (1H, m, H-3), 5.70 (1H, d, *J* = 2.4 Hz, H-6 or H-8), 5.74 (1H, d, *J* = 2.4 Hz, H-8 or H-6), 6.26 (2H, s, H-2′ and H-6′); EIMS: *m*/*z* 432.2 $[M]^+$; EIHRMS *m*/*z*: 432.1776 ($[M]^+$, calcd for $C_{23}H_{28}O_8$: 432.1784).

3-*O*-Decanoyl-(−)-EGC (3d). 31.0% yield. $[\alpha]_D^{20}$ −53.6° (EtOH, *c* 0.63); IR ν_{max} (KBr) 3418, 2929, 2860, 1714, 1633, 1516, 1476, 1324, 1260, 1191, 1144, 1103, 1010, 824, 719 cm^{-1}; ^{1}H NMR (270 MHz, CD_3OD) δ: 0.69 (3H, t, *J* = 6.6 Hz, –$COCH_2CH_2(CH_2)_6C\underline{H}_3$), 1.05 (12H, m, –$COCH_2CH_2(C\underline{H}_2)_6CH_3$), 1.24 (2H, m, –$COCH_2C\underline{H}_2(CH_2)_6CH_3$), 1.94 (2H, t, *J* = 7.6 Hz, –$COC\underline{H}_2CH_2$–$(CH_2)_6CH_3$), 2.58 (1H, AB of ABX, *J* = 2.1 and 17.6 Hz, H-4), 2.71 (1H, AB of ABX, *J* = 4.6 and 17.6 Hz, H-4), 4.68 (1H, s, H-2), 5.14 (1H, m, H-3), 5.75 (1H, d, *J* = 1.9 Hz, H-6 or H-8), 5.75 (1H, d, *J* = 1.9 Hz, H-8 or H-6), 6.27 (2H, s, H-2′ and H-6′); FABMS: *m*/*z* 461.22 $[M+H]^+$; FABHRMS *m*/*z*: 461.2178 ($[M+H]^+$, calcd for $C_{25}H_{33}O_8$: 461.2175).

3-*O*-Dodecanoyl-(−)-EGC (3e). 35.6% yield. $[\alpha]_D^{20}$ −40.8° (EtOH, *c* 0.98); IR ν_{max} (KBr) 3422, 2926, 2857, 1702, 1634, 1515, 1462, 1307, 1184, 1143, 1013, 825, 724 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ: 0.89 (3H, t, *J* = 6.8 Hz, –$COCH_2CH_2(CH_2)_8C\underline{H}_3$), 1.20–1.27 (16H, m, –$COCH_2CH_2(C\underline{H}_2)_8CH_3$), 1.43 (2H, m, –$COCH_2C\underline{H}_2(CH_2)_8CH_3$), 2.77 (1H, AB of ABX, *J* = 2.2 and 17.4 Hz, H-4), 2.90 (1H, AB of ABX, *J* = 4.5 and 17.4 Hz, H-4), 5.33 (1H, m, H-3), 5.90 (1H, d, *J* = 2.3 Hz, H-6 or H-8), 5.93 (1H, d, *J* = 2.3 Hz, H-8 or H-6), 6.46 (2H, s, H-2′ and H-6′); FABMS: *m*/*z* 489.3 $[M+H]^+$; FABHRMS *m*/*z*: 489.2465 ($[M+H]^+$, calcd for $C_{27}H_{37}O_8$: 489.2488).

3-*O*-Myristoyl-(−)-EGC (3f). 44.7% yield. $[\alpha]_D^{19}$ −39.4° (EtOH, *c* 1.1); IR ν_{max} (KBr) 3433, 2925, 2857, 1718, 1630, 1515, 1467, 1317, 1184, 1144, 1098, 1018, 821, 724 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ: 0.89 (3H, t, *J* = 6.7 Hz, –$COCH_2CH_2(CH_2)_{10}C\underline{H}_3$), 1.20–1.27 (20H, m, –$COCH_2CH_2$–$(C\underline{H}_2)_{10}CH_3$), 1.43 (2H, m, –$COCH_2C\underline{H}_2(CH_2)_{10}CH_3$), 2.19 (2H, t, *J* = 7.3 Hz, –$COC\underline{H}_2$–$(CH_2)_{10}CH_3$), 2.77 (1H, AB of ABX, *J* = 2.2 and 17.5 Hz, H-4), 2.90 (1H, AB of ABX, *J* = 2.2 and 17.5 Hz, H-4), 5.33 (1H, m, H-3), 5.90 (1H, d, *J* = 2.3 Hz, H-6 or H-8), 5.94 (1H, d, *J* = 2.3, H-8 or H-6), 6.46 (2H, s, H-2′ and H-6′); EIMS: *m*/*z* 516.3 $[M]^+$; EIHRMS *m*/*z*: 516.2692 ($[M]^+$, calcd for $C_{29}H_{40}O_8$: 516.2723).

3-*O*-Palmitoyl-(−)-EGC (3g). 44.9% yield. $[\alpha]_D^{22}$ −32.5° (EtOH, *c* 2.51); IR ν_{max} (KBr) 3423, 2924, 2852, 1718, 1627, 1522, 1466, 1344, 1253, 1184, 1144, 1093, 1016, 825, 719 cm^{-1}; ^1H NMR (270 MHz, CD_3OD) δ: 0.91 (3H, t *J* = 6.9 Hz, –$COCH_2CH_2(CH_2)_{12}C\underline{H}_3$), 1.01–1.37 (24H, m, –$COCH_2CH_2(C\underline{H}_2)_{12}CH_3$), 1.44 (2H, m, –$COCH_2C\underline{H}_2(CH_2)_{12}CH_3$), 2.19 (2H, t, *J* = 6.8 Hz, –$COC\underline{H}_2CH_2(CH_2)_{12}CH_3$), 2.76 (1H, brd, *J* = 17.8 Hz, H-4), 2.89 (1H, brd, *J* = 17.8 Hz, H-4), 4.97 (1H, s, H-2), 5.31 (1H, m, H-3), 5.90 (1H, brs, H-6 or H-8), 5.92 (1H, brs, H-8 or H-6), 6.45 (2H, s); EIMS: *m*/*z* 544.3 $[M]^+$; EIHRMS *m*/*z*: 544.2979 ($[M]^+$, calcd for $C_{31}H_{44}O_8$: 544.3036).

3-*O*-Stearoyl-(−)-EGC (3h). 41.7% yield. $[\alpha]_D^{21}$ −25.8° (EtOH, *c* 0.91); IR ν_{max} (KBr) 3420, 2925, 2851, 1718, 1618, 1541, 1458, 1312, 1180, 1141, 1085, 1016, 822, 711 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ: 0.94 (3H, t, *J* = 6.9 Hz, –$COCH_2CH_2(CH_2)_{14}C\underline{H}_3$), 1.33 (28H, m, –$COCH_2CH_2(C\underline{H}_2)_{14}CH_3$), 1.48 (2H, m, –$COCH_2C\underline{H}_2(CH_2)_{14}CH_3$), 2.24 (2H, t, *J* = 7.1 Hz, –$COC\underline{H}_2CH_2(CH_2)_{14}CH_3$), 2.82 (1H, AB of ABX, *J* = 2.3 and 17.4 Hz, H-4), 2.95 (1H, AB of ABX, *J* = 4.7 and 17.4 Hz, H-4), 4.93 (1H, s, H-2), 5.38 (1H, m, H-3), 5.96 (1H, d, *J* = 2.3 Hz, H-6 or H-8), 5.99 (1H, d, *J* = 2.3 Hz, H-8 or H-6), 6.52 (2H, s, H-2′ and H-6′); FABMS: *m*/*z* 573.3 $[M+H]^+$. FABHRMS *m*/*z*: 573.3464 ($[M+H]^+$, calcd for $C_{33}H_{49}O_8$: 573.3427).

[3(2′*S*),4*S*]-3-(2-Methyloctanoyl)-4-benzyl-2-oxazolidinone (10) and [3(2′*R*),4*R*]-3-(2-methyloctanoyl)-4-benzyl-2-oxazolidinone (11). (4*S*)-3-(**8**) (3.5 g) and (4*R*)-3-(octanoyl)-4-benzyl-2-oxazolidinone (**9**) (2.5 g) were converted to [3(2′*S*),4*S*]-3- (**10**) (2.9 g, 85% yield) and [3(2′*R*),4*R*]-3-(2-methyloctanoyl)-isomer (**11**) (1.6 g, 61% yield), respectively, through diastereoselective α-methylation with MeI and sodium bis(trimethylsilyl)amide followed by chromatographic purification according to the reported procedure.[14,15]

[3(2′*S*),4*S*]-isomer (10). $[\alpha]_D^{20}$ +68.2° (MeOH, *c* 0.80); IR ν_{max} (neat) 2930, 1778, 1693, 1454, 1385, 1348, 1196, 1099, 1016, 972, 702 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ: 0.88 (3H, t, *J* = 7.0 Hz), 1.22 (3H, d, *J* = 6.8 Hz), 1.27 (8H, m), 1.71 (1H, m), 2.77 (1H, dd, *J* = 9.5 and 13.2 Hz), 3.27 (1H, dd, *J* = 3.2 and 13.2 Hz), 3.70 (1H, m), 4.07–4.23 (2H, m), 4.62–4.72 (1H, m), 7.19–7.35 (5H, m); EIMS: *m*/*z* 317 $[M]^+$; EIHRMS *m*/*z*: 317.1988 ($[M]^+$, calcd for $C_{19}H_{27}NO_3$: 317.1991).

[3(2′*R*),4*R*]-isomer (11). $[\alpha]_D^{20}$ −63.2° (MeOH, *c* 1.1); IR ν_{max} (neat) 2930, 1780, 1697, 1454, 1385, 1348, 1195, 1099, 1016, 972, 702 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ: 0.87 (3H, t, *J* = 7.0 Hz), 1.22 (3H, d, *J* = 7.0 Hz), 1.27 (8H, m), 1.69 (1H, m), 2.76 (1H, dd, *J* = 9.5 and 13.2 Hz), 3.26 (1H, dd, *J* = 3.2 and 13.2 Hz), 3.70 (1H, m), 4.07–4.23 (2H, m), 4.62–4.71 (1H, m), 7.19–7.35 (5H, m); EIMS: *m*/*z* 317.0 $[M]^+$; EIHRMS *m*/*z*: 317.1981 ($[M]^+$, calcd for $C_{19}H_{27}NO_3$: 317.1991).

***(S)*-2-(12) and *(R)*-2-methyloctanoic acid (13).** Compound **10** or **11** (each 1.2 mmol) was dissolved in a mixture of tetrahydrofuran (18 mL) and dist. Water (6 mL) containing $LiOH{\cdot}H_2O$ (2.0 mmol) and 30% H_2O_2 (9.9 mmol). After stirring at rt for 4 h, the reaction was quenched with 1 M $Na_2S_2O_3$. The water layer was adjusted to pH 1 with 10% HCl and extracted with Et_2O. The Et_2O layer was washed with satd NaCl, dried and concentrated in vacuo to give a brownish oily acid. It was purified by SiO_2 column chromatography with AcOEt/*n*-hexane (1:4) followed by vacuum distillation at 120–123 °C.

***(S)*-2-Methyloctanoic acid (12).** 69.0% yield. $[\alpha]_D^{20}$ +16.2° (MeOH, *c* 1.1); IR ν_{max} (neat) 3100, 2930, 1713, 1467, 1418, 1238, 941 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ: 0.88 (3H, t, *J* = 6.8 Hz), 1.15 (3H, d, *J* = 1.68 Hz), 1.21–1.39 (10H, m), 2.45 (1H, m); EIMS: *m*/*z*: 158.1 $[M]^+$; EIHRMS *m*/*z*: 158.1309 ($[M]^+$, calcd for $C_9H_{18}NO_2$: 158.1307).

***(R)*-2-methyloctanoic acid (13).** 53.5% $[\alpha]_D^{20}$ −15.3° (MeOH, *c* 1.6); IR ν_{max} (neat) 3100, 2930, 1710, 1465, 1417, 1236, 941 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ: 0.85 (3H, t, *J* = 6.8 Hz), 1.17 (3H, d, *J* = 7.0 Hz), 1.21–1.39 (10H, m), 2.45 (1H, m); EIMS *m*/*z* 158.1 $[M]^+$; EIHRMS *m*/*z*: 158.1303 ($[M]^+$, calcd for $C_9H_{18}NO_2$: 158.1307).

General procedure for the synthesis of (−)-EGCs possessing a 2-methyl-substituted acyl group

(*RS*)-2-Methyloctanoic acid,[21] (*RS*)-2-methyldecanoic acid,[21] **12** or **13** (0.360 mmol) was converted to the corresponding acid chloride with $SOCl_2$. It was then dissolved in a tetrahydrofuran solution (1 mL) containing (−)-EGC (0.327 mmol) and trifluoroacetic acid (0.65 mmol), and the solution was stirred for 24 h under an Ar gas. After concentration of the reaction mixture, the

residue was extracted with AcOEt, which was washed with satd. NaCl. The organic layer was dried and concentrated in vacuo to give a residue, which was purified by preparative HPLC, followed by freeze-drying, yielding a white powder.

3-*O*-[(*RS*)-2-Methyloctanoyl]-(−)-EGC (4). 22.0% yield. IR ν_{max} (KBr) 3436, 2931, 2861, 1718, 1637, 1508, 1467, 1343, 1190, 1143, 1102, 1008, 879, 826, 726 cm^{-1}. ^1H NMR (270 Hz, CD_3OD) δ: 0.81 (3H×0.5, t, $J=6.9$ Hz, –$COCH(CH_3)CH_2(CH_2)_4C\underline{H}_3$), 0.82 (3H×0.5, t, $J=6.9$ Hz, –$COCH(CH_3)CH_2(CH_2)_4C\underline{H}_3$), 0.88 (3H×0.5, d, $J=6.8$ Hz, –$COCH(C\underline{H}_3)CH_2(CH_2)_4CH_3$), 0.92 (3H×0.5, d, $J=7.3$ Hz, –$COCH(C\underline{H}_3)CH_2(CH_2)_4CH_3$), 0.9–1.35 (10H, m, –$COCH(CH_3)C\underline{H}_2(C\underline{H}_2)_4CH_3$), 2.25 (1H, m, –$COC\underline{H}(CH_3)CH_2(CH_2)_4CH_3$), 2.75 (1H, brd, $J=17.3$ Hz, H-4), 2.85 (1H, AB of ABX, $J=4.3$ and 17.3 Hz, H-4), 5.25 (1H, m, H-3), 5.88 (2H, m, H-6 and H-8), 6.40 (2H, s, H-2′ and H-6′); FABMS: *m/z* 447.20 $[M+H]^+$; FABHRMS *m/z*: 447.2036 ($[M+H]^+$, calcd for $C_{24}H_{31}O_8$: 447.2019).

3-*O*-[(*RS*)-2-Methyldecanoyl]-(−)-EGC (5). 16.7% yield. IR ν_{max} (KBr) 3436, 2931, 2861, 1700, 1616, 1522, 1459, 1342, 1265, 1195, 1144, 1098, 1015, 824, 723 cm^{-1}. ^1H NMR (CD_3OD) δ: 0.81–0.98 (6H, m, –$COCH(C\underline{H}_3)CH_2(CH_2)_6C\underline{H}_3$), 1.01–1.41 (14H, m, –$COCH(CH_3)C\underline{H}_2(C\underline{H}_2)_6CH_3$), 2.22–2.23 (1H, m –$COC\underline{H}(CH_3)CH_2(CH_2)_6CH_3$), 2.79 (1H,AB of ABX, $J=5.4$ and 13.5 Hz, H-4), 2.88 (1H, AB and ABX $J=4.3$ and 17.6 Hz, H-4), 4.87 (1H, s, H-2), 5.27 (1H, m, H-3), 5.90 (1H, d, $J=1.90$ Hz, H-6 and H-8), 5.92 (1H, d, $J=2.4$ Hz, H-8 and H-6), 6.46 (2H, s, H-2′ and H-6′); FABMS: *m/z*: 475.24 $[M+H]^+$; FABHRMS *m/z*: 475.2355 ($[M+H]^+$, calcd for $C_{26}H_{35}O_8$: 475.2332).

3-*O*-[(*S*)-2-Methyloctanoyl]-(−)-EGC (6). 13.4% yield. $[\alpha]_D^{27}$ −23.2° (EtOH, *c* 0.11); IR ν_{max} (KBr) 1706, 1608, 1458, 1299, 1145, 826; ^1H NMR (500 MHz, CD_3OD) δ: 0.84 (3H, t, $J=6.9$ Hz, –$COCH(CH_3)CH_2(CH_2)_4C\underline{H}_3$), 0.91 (3H, d, $J=7.3$ Hz, –$COCH(C\underline{H}_3)CH_2(CH_2)_4CH_3$), 0.9–1.35 (10H, m, –$COCH(CH_3)C\underline{H}_2(C\underline{H}_2)_4CH_3$), 2.34 (1H, m, –$COC\underline{H}(CH_3)CH_2(CH_2)_4CH_3$), 2.75 (1H, brd, $J=17.2$ Hz, H-4), 2.85 (1H, AB of ABX, $J=3.7$ and 17.2 Hz, H-4), 5.29 (1H, m, H-3), 5.94 (2H, m, H-6 and H-8), 6.57 (2H, s, H-2′ and H-6′); FABMS: *m/z* 447.21 $[M+H]^+$; FABHRMS *m/z*: 447.2036 ($[M+H]^+$, calcd for $C_{24}H_{31}O_8$: 447.2019).

3-*O*-[(*R*)-2-Methyloctanoyl]-(−)-EGC (7). 8.6% yield. $[\alpha]_D^{28}$ −21.7° (EtOH, *c* 0.03); IR ν_{max} (KBr) 1706, 1608, 1458, 1299, 1145, 826; ^1H NMR (400 MHz, CD_3OD) δ: 0.77 (3H, t, $J=7.0$ Hz, –$COCH(CH_3)CH_2(CH_2)_4C\underline{H}_3$), 0.87 (3H, d, $J=7.2$ Hz, –$COCH(C\underline{H}_3)CH_2(CH_2)_4CH_3$), 0.9–1.35 (10H, m, –$COCH(CH_3)C\underline{H}_2(C\underline{H}_2)_4CH_3$), 2.17 (1H, q, $J=7.2$ Hz, –$COC\underline{H}(CH_3)CH_2(CH_2)_4CH_3$), 2.71 (1H, AB of ABX, $J=2.0$ and 17.6 Hz, H-4), 2.80 (1H, AB of ABX, $J=4.4$ and 17.6 Hz, H-4), 4.81 (1H, s, H-2), 5.19 (1H, brs, H-3), 5.82 (1H, d, $J=2.2$ Hz, H-6 or H-8), 5.84 (1H, d, $J=2.2$ Hz, H-8 or H-6), 6.40 (2H, s, H-2′ and H-6′); FABMS: *m/z* 445.19 $[M-H]^-$, FABHRMS *m/z*: 445.1859 ($[M-H]^-$, calcd for $C_{24}H_{29}O_8$: 445.1863).

Conformational analysis

The conformations of **11** and **16** were analyzed by the software MacSPARTAN Pro (version 2.0, Wavefunction, Inc.) using the molecular mechanics method with MMFF94 force field.[19] The global minimum-energy conformation of each compound was generated by repeating rotations (360° at 60° intervals) of rotatable ester C–C and C–O bonds in the side chain followed by optimization.

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