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Synthesis and Biological Evaluation of the 12,12-Dimethyl Derivative of Aplog-1, an Anti-Proliferative Analog of Tumor-Promoting Aplysiatoxin

Yu NAKAGAWA,[1,2] Masayuki KIKUMORI,[1] Ryo C. YANAGITA,[1,3] Akira MURAKAMI,[1] Harukuni TOKUDA,[4] Hiroshi NAGAI,[5] and Kazuhiro IRIE[1,†]

[1]*Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan*
[2]*Synthetic Cellular Chemistry Laboratory, Advanced Science Institute, RIKEN (The Institute of Physical and Chemical Research), Wako 351-0198, Japan*
[3]*Department of Applied Biological Science, Faculty of Agriculture, Kagawa University, Kagawa 761-0195, Japan*
[4]*Graduate School of Medical Science, Kanazawa University, Kanazawa 920-8640, Japan*
[5]*Tokyo University of Marine Science and Technology, Minato-ku, Tokyo 108-8477, Japan*

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Aplog-1 is a unique analog of tumor-promoting aplysiatoxin that inhibits tumor-promotion by phorbol diesters and proliferation of tumor cells. While the structural features relevant to the biological activities of Aplog-1 remain to be identified, recent studies by us have suggested that local hydrophobicity around the spiroketal moiety of Aplog-1 is a crucial determinant of its anti-proliferative activity. This hypothesis led us to design 12,12-dimethyl-Aplog-1 (3), in which a hydrophobic geminal dimethyl group is installed proximal to the spiroketal moiety to improve biological potency. As expected, 3 was more effective than Aplog-1 in inhibiting cancer cell growth and binding to protein kinase Cδ, a putative receptor responsible for the biological response of Aplog-1. Moreover, an induction test on Epstein-Barr virus early antigen demonstrated 3 to be a better anti-tumor promoter than Aplog-1. These results indicate that 3 is a superior derivative of Aplog-1, and thus a more promising lead for anti-cancer drugs.

Key words: Aplog-1; aplysiatoxin; bryostatin; protein kinase C; tumor promoter

Aplysiatoxin (ATX, Fig. 1) is a polyacetate-type tumor promoter isolated from the digestive gland of the sea hare *Stylocheilus longicauda*.[1)] ATX exhibited tumor-promoting activity *in vivo* comparable to other naturally-occurring tumor promoters, such as 12-*O*-tetradecanoylphorbol 13-acetate (TPA) and teleocidin B-4, in the two-stage mouse skin carcinogenesis system.[2)] The mode of action of these tumor promoters has been proposed to arise from activation of protein kinase C (PKC).[3,4)] PKC is a family of serine/threonine kinases that play crucial roles in cellular signal transduction, including proliferation, differentiation, and apoptosis.[5,6)] ATX, TPA, and teleocidin B-4 bind competitively to the regulatory domain of PKC despite the differences in their chemical structures (Fig. 1).[7)] While the tumor-promoting activity of phorbol diesters and teleocidins correlates well with their affinity for PKC, discrepancies exist in ATX-related compounds. An example is debromo-ATX, which retains affinity for PKC but shows reduced tumor-promoting activity as compared to ATX.[8,9)] This observation has been interpreted to result from decreased hydrophobicity due to the loss of a bromine atom, suggesting that the tumor-promoting activity of ATX is highly dependent on hydrophobicity while its binding to PKC is not.

On the basis of this finding, we recently developed a simple and less hydrophobic analog of ATX (Aplog-1,[10)] Fig. 2) as a new PKC activator with much reduced tumor-promoting activity, like bryostatin 1 (Bryo-1, Fig. 1), a potent PKC activator without tumor-promoting activity isolated from the marine bryozoan *Bugula neritina*.[11–13)] Aplog-1 inhibited tumor-promotion by TPA and proliferation of human cancer cell lines despite retaining the skeleton of tumor-promoting ATX. In subsequent efforts to identify the structural features relevant to the unique biological profile of Aplog-1, we synthesized 18-deoxy derivatives of Aplog-1 (**1**, **2**).[10,14)] Biological evaluation of them revealed the importance of the geminal dimethyl group at position 6 for anti-proliferative activity and PKC binding of Aplog-1. This finding suggests that the spiroketal moiety of Aplog-1 is involved in the hydrophobic interaction with PKC, suggesting the possibility of enhancing the biological potency of Aplog-1 by increasing local hydrophobicity around the spiroketal moiety. This led us to design 12,12-dimethyl-Aplog-1 (**3**, Fig. 2), in which a hydrophobic geminal dimethyl group is installed proximal to the spiroketal moiety. In this paper, we report the synthesis, PKC binding, anti-proliferative activity, and tumor-promoting activity of **3**, which proved to be superior to Aplog-1 in three biological assays, suggesting that it is a promising lead for anti-cancer drugs.

Results and Discussion

12,12-Dimethyl-Aplog-1 (**3**) was synthesized from known bromide **4**,[15)] as shown in Scheme 1. Substitution

† To whom correspondence should be addressed. Tel: +81-75-753-6281; Fax: +81-75-753-6284; E-mail: irie@kais.kyoto-u.ac.jp

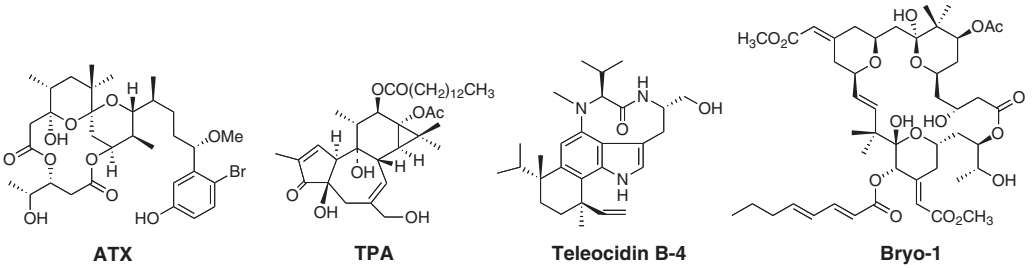


Fig. 1. Structures of Aplysiatoxin (ATX), 12-*O*-Tetradecanoylphorbol 13-acetate (TPA), Teleocidin B-4, and Bryostatin 1 (Bryo-1).

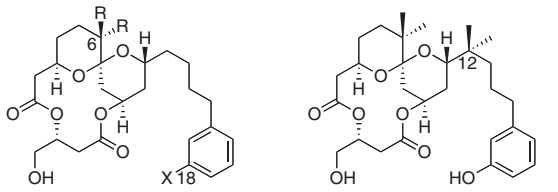


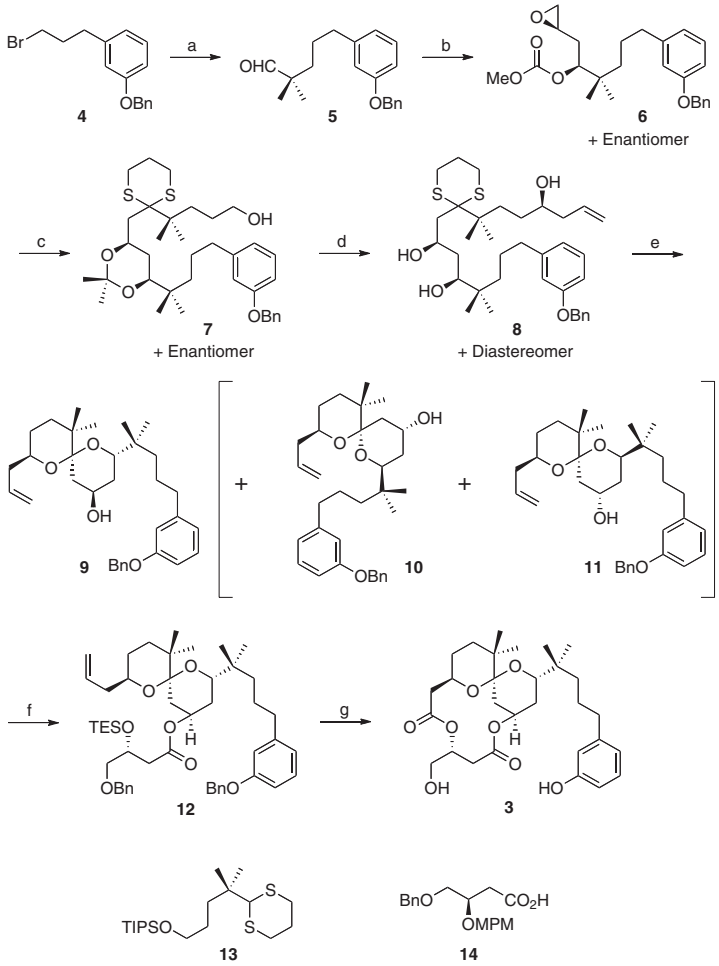
Fig. 2. Structures of Aplog-1, Its 18-Deoxy-derivatives (**1**, **2**), and 12,12-Dimethyl-Aplog-1 (**3**).

with ethyl isobutyrate and conversion of the resulting ester to an aldehyde provided **5**. Asymmetric Brown's allylation[16] of **5** produced an (*S*)-homoallyl alcohol with an ee of 67% (Supplemental Fig. 1; see *Biosci. Biotechnol. Biochem.* Web site). Attempts to improve the enantioselectivity of the homoallyl alcohol employing Keck's[17] and Maruoka's[18] allylations resulted in no reaction, probably due to steric crowding at the aldehyde group. Stereoselective iodocarbonate cyclization[19] of the homoallyl alcohol generated a cyclic carbonate, which was converted to epoxide **6** by methanolysis. Due to steric hindrance by the geminal dimethyl group, the methyl carbonate of **6** was less susceptible to methanolysis with the use of K_2CO_3 in MeOH at room temperature. Attempts to cleave the methyl carbonate by methanolysis under reflux conditions or by hydrolysis using aqueous NaOH in MeOH resulted in the decomposition of **6**. Hence, **6** was used for the coupling reaction with dithiane **13**,[10] though the carbonate group is generally sensitive to strong basic conditions. As expected, the coupling reaction produced several products derived from side reactions at the carbonate site. Since most of these by-products were anticipated to contain ester or carbonate moieties, reduction with $LiAlH_4$ was performed to convert them into a single diol. After *O*-isopropylidenation of the diol followed by desilylation, **7** was successfully obtained in 69% yield in 4 steps from **6**. Oxidation of the alcohol of **7**, followed by Maruoka's asymmetric allylation[18] and acid hydrolysis of the isopropylidene acetal, produced an inseparable mixture of homoallyl alcohol **8** and its diastereomer. Hydrolysis of the dithiane moiety of the diastereomeric mixture with $Hg(ClO_4)_2 \cdot 6H_2O$ produced three spiroketals (**9**, **10**, **11**), whose configurations were determined by nuclear Overhauser enhancement (NOE), as shown in Supplemental Fig. 2. Yamaguchi esterification[20] of desired spiroketal **9** with known carboxylic acid **14**,[10] followed by switching of the protecting group from the 4-methoxyphenylmethyl (MPM) group to a triethylsilyl (TES) group, provided mono-ester **12**. After oxidative cleavage of the olefin group and deprotection of the TES group, lactonization was performed by the Yamaguchi method.[20] Final deprotection of the two benzyl groups provided target compound **3** in 22 steps, with an overall yield of 2.4%.

Compound **3** was initially evaluated for its ability to bind PKCδ. Several lines of evidence indicate that PKCδ is an important isozyme responsible for the anti-cancer actions of Bryo-1.[21,22,24] We found previously that Aplog-1 displayed binding and activation profiles for PKCδ similar to those of Bryo-1, and that its anti-proliferative activity was closely correlated with its affinity for PKCδ.[10,14] The affinity of **3** for PKCδ was measured in a competition binding assay with [^{3}H]phorbol 12,13-dibutyrate (PDBu).[25] Compound **3** exhibited strong affinity for PKCδ ($K_i = 5.9$ nM), about 2.5 times higher than that of Aplog-1 ($K_i = 15$ nM). This increase as compared to Aplog-1 indicates that as expected, the geminal dimethyl group at position 12 of **3** makes a positive contribution to hydrophobic interaction with PKCδ.

Encouraged by this result, we evaluated the anti-proliferative activities of **3** against a panel of 39 human cancer cell lines, as described previously.[26] Growth inhibitory activity was expressed as the concentration required to inhibit cell growth by 50% as compared to an untreated control, GI_{50} (M). The results for the cell lines, in which Aplog-1 showed significant activities with GI_{50} values of less than 10^{-5} M, are listed in Table 1 (the rest are provided in Supplemental Table 1). As expected based on the results for PKCδ binding, **3** was more potent than Aplog-1 in most cancer cell lines. The mean-graph midpoint (MG-MID) values for Aplog-1 and **3** were -4.98 and -5.12 respectively. This correlation between PKCδ binding and anti-proliferative activities of **3** further confirms that PKCδ is the receptor responsible for the anti-proliferative activity of Aplog-related compounds.

The most critical point in developing derivatives of Aplog-1 is to confirm that the structural modifications can be made without increasing tumor-promoting activity, since Aplog-1 possesses the skeleton of tumor-promoting ATX. Especially in the case of **3**, attachment of the geminal dimethyl group resulted in increased hydrophobicity, which is prone to enhance the tumor-promoting activities of ATX-related compounds.[8,9] Hence, we evaluated the tumor-promoting and its inhibiting activities of **3** by testing to determine



Scheme 1. Synthesis of **3**.

(a) (1) Ethyl isobutyrate, LDA, THF, HMPA; (2) $LiBH_4$, THF; (3) SO_3·Pyr, NEt_3, DMSO, CH_2Cl_2; 88% in 3 steps. (b) (1) AllylMgBr, (−)-Ipc_2BOMe, THF, 67% ee; (2) $(Boc)_2O$, NaHMDS, THF; (3) IBr, CH_2Cl_2, Toluene; (4) K_2CO_3, MeOH; 72% in 4 steps. (c) (1) **13**, *n*-BuLi, THF; (2) $LiAlH_4$, THF; (3) 2,2-Dimethoxypropane, CSA, CH_2Cl_2; (4) TBAF, THF; 69% in 4 steps. (d) (1) SO_3·Pyr, NEt_3, DMSO, CH_2Cl_2; (2) $TiCl_4$, $Ti(OiPr)_4$, (*S*)-BINOL, Ag_2O, $AllylSnBu_3$, CH_2Cl_2; (3) TsOH·H_2O, H_2O, CH_3CN, THF; 63% in 3 steps. (e) $Hg(ClO_4)_2$·$6H_2O$, MeCN, CH_2Cl_2, H_2O; 42% for **9**, 22% for **10**, 18% for **11**. (f) (1) **14**, TCBCl, NEt_3, DMAP, Toluene; (2) DDQ, H_2O, CH_2Cl_2; (3) TESCl, Imidazole, THF; 50% in 3 steps. (g) (1) $KMnO_4$, $NaIO_4$, *t*-BuOH, pH 7 buffer; (2) HF·Pyr, Pyridine, THF; (3) TCBCl, NEt_3, DMAP, Toluene; (4) H_2, 10% Pd-C, MeOH, $CHCl_3$, 41% in 4 steps

Table 1. Log GI_{50} Values for Aplog-1 and **3** against a Subset of 39 Human Cancer Cell Lines

Cancer type	Cell line	log GI_{50} (log M)	
		Aplog-1[a]	**3**
Breast	HBC-4	−6.33	−6.67
Breast	MDA-MB-231	−5.61	−5.92
CNS	SF-295	−5.06	−5.32
Colon	HCC2998	−5.43	−6.06
Lung	NCI-H460	−5.60	−6.05
Lung	A549	−5.32	−5.51
Melanoma	LOX-IMVI	−5.74	−6.06
Stomach	St-4	−5.55	−6.20
Stomach	MKN45	−5.33	−5.33

[a]Cited from reference 11.

whether Epstein-Barr virus early antigen (EBV-EA) would be induced in Raji cells.[27] EBV is activated by treatment with tumor promoters to produce EA, which can be detected by an indirect immunofluorescence technique.[28,29] EBV-EA-inducing activity is expressed as the percentage of EA-positive cells (Figs. 3 and 4). Compared to ATX which has significant EA-inducing activity, Aplog-1 has been found to exhibit weak EA-induction by itself (Fig. 3) and suppress markedly the production of EA induced by TPA (Fig. 4).[10] Only weak EA-induction (9.0% and 7.1% at 10^{-6} and 10^{-7} M respectively) and significant suppression of TPA-induced EA production (5.0% and 7.2% at 10^{-6} and 10^{-7} M respectively) were observed, suggesting that **3** is a better anti-tumor promoter than Aplog-1. Although our previous study using **2** found no correlation between inhibition of TPA-induced EA production and suppression of proliferation of tumor cells,[14] introduction of the geminal dimethyl group into position 12 of Aplog-1 was found to enhance both activities.

In conclusion, 12,12-dimethyl-Aplog-1 (**3**) was developed as a superior derivative of Aplog-1. Three biological assays demonstrated that **3** was more effective than Aplog-1 in binding to PKCδ, inhibiting cancer cell growth and suppressing TPA-induced EA production. These results collectively indicate that **3** is a more promising lead for anti-cancer drugs, and that the introduction of a hydrophobic group at pertinent positions of Aplog-1 can enhance anti-proliferative activity without increasing tumor-promoting activity.

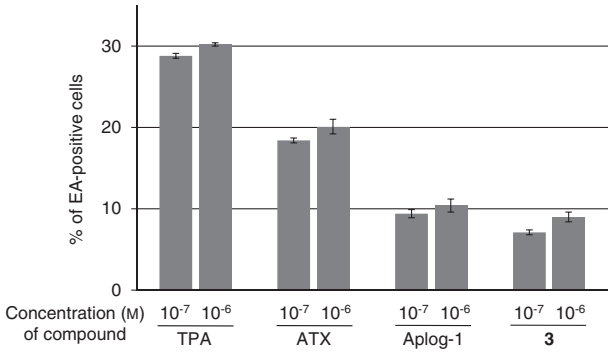


Fig. 3. EBV-EA Production Induced by TPA, ATX, Aplog-1, and **3**.
Percentages of EA-positive cells are shown. Sodium *n*-butyrate (4 mM) was added to all samples to enhance the sensitivity of the Raji cells. Only 0.1% of the cells were positive for EA at 4 mM of sodium *n*-butyrate. The final concentration of DMSO was 0.4%. Cell viability exceeded 50% in all experiments, except for ATX at 10^{-6} M (40%). Error bars represent standard error of the mean ($n = 3$).

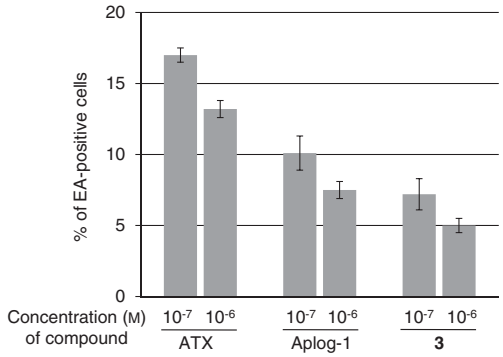


Fig. 4. Inhibition of TPA-Induced EBV-EA Production by ATX, Aplog-1, and **3**.
Percentages of EA-positive cells in the presence of TPA ($10^{-7.5}$ M) are shown. At $10^{-7.5}$ M of TPA, 30 ± 0.6% of cells were positive for EA. Sodium *n*-butyrate (4 mM) was added to all samples to enhance the sensitivity of the Raji cells. Only 0.1% of the cells were positive for EA at 4 mM of sodium *n*-butyrate. The final concentration of DMSO was 0.4%. Cell viability exceeded 50% in all experiments, except for ATX at 10^{-6} M (40%). Error bars represent standard error of the mean ($n = 3$).

Efforts to identify additional superior derivatives of Aplog-1 are underway.

Experimental

General remarks. The following spectroscopic and analytical instruments were used: UV, UV-2200A (Shimadzu, Kyoto, Japan); Digital Polarimeter, DIP-1000 (Jasco, Tokyo, Japan); ^{1}H NMR, AVANCE I 400 and AVANCE III 500 (ref. TMS, 296 K, Bruker, Germany); HPLC, Model 600E with a Model 2487 UV detector (Waters, Tokyo, Japan); (HR) EI-MS, JMS-600H (JEOL, Tokyo, Japan). HPLC was carried out on a YMC packed A-023 (silica gel, 10 mm i.d. × 250 mm) column (Yamamura Chemical Laboratory, Kyoto, Japan). Wako gel C-200 (silica gel, Wako Pure Chemical Industries, Osaka, Japan) and YMC A60-350/250 gel (ODS, Yamamura Chemical Laboratory, Kyoto, Japan) were used for column chromatography. [^{3}H]PDBu (16.3 Ci/mmol) was purchased from PerkinElmer Life Sciences Research Products (Boston, MA, USA). All other chemicals and reagents were purchased from chemical companies, and were used without further purification.

Synthesis of ***5***. To a solution of diisopropylamine (3.4 mL, 26.2 mmol, 2.4 equiv.) in THF (30 mL) was added 1.6 M *n*-BuLi in hexane (15 mL, 24.0 mmol, 2.2 equiv.) at −78 °C. After stirring at −78 °C for 5 min, the mixture was warmed to 0 °C, and stirring was continued for 10 min. The resulting lithium diisopropylamide (LDA) solution was recooled to −78 °C, and ethyl isobutyrate (2.9 mL, 21.8 mmol, 2.0 equiv.) was slowly added. The mixture was stirred at −78 °C for 20 min, and then a solution of **4**[12] (3.30 g, 10.9 mmol) and hexamethylphosphoramide (HMPA, 2.0 mL) in THF (5 mL) was added. The reaction mixture was stirred at −78 °C for 4 h, and stirring was continued in a cold room for 15 h. The reaction was quenched with saturated aq. NH_4Cl (50 mL), and the mixture was extracted with EtOAc (50 mL × 3). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, 1% → 3% EtOAc/hexane) to afford the crude ester (4.54 g). To a solution of the ester (4.54 g) in THF (24 mL) was added $LiBH_4$ (650 mg, 29.8 mmol) at rt. The reaction mixture was stirred at rt for 18 h, and the reaction was quenched with 0.5 N HCl (20 mL). The resulting mixture was extracted with EtOAc (100 mL × 3). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, 5% → 10% EtOAc/hexane) to afford the alcohol (3.74 g). To a solution of the alcohol (3.74 g) and NEt_3 (7.0 mL, 50.4 mmol) in CH_2Cl_2 (150 mL) were added DMSO (27 mL, 378 mmol) and SO_3·pyridine (6.0 g, 37.8 mmol) at rt. The mixture was stirred at rt for 18 h, and the reaction was quenched with saturated aq. NH_4Cl (100 mL). The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (100 mL × 2). The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, 3% EtOAc/hexane) to afford **5** (2.85 g, 9.63 mmol, 88% in 3 steps) as a clear oil. ^{1}H NMR: (400 MHz, $CDCl_3$, 0.067 M) δ 1.02 (6H, s), 1.47–1.56 (4H, m), 2.57 (2H, t, $J = 7.1$ Hz), 5.05 (2H, s), 6.76–6.82 (3H, m), 7.19 (1H, dt, $J = 7.4$, 0.7 Hz), 7.32–7.45 (5H, m), 9.42 (1H, s) ppm. ^{13}C NMR: (100 MHz, $CDCl_3$, 0.067 M) δ 21.30 (2C), 25.93, 36.35, 36.73, 45.75, 69.93, 112.02, 115.17, 121.06, 127.51 (2C), 127.92, 128.57 (2C), 129.34, 137.13, 143.61, 158.90, 206.26 ppm. HR-EIMS: m/z ([M^+]): Calcd. for $C_{20}H_{24}O_2$: 296.1776, Found: 296.1764.

Synthesis of ***6***. To a solution of (−)-*B*-methoxydiisopinocampheylborane (Ipc_2BOMe, 3.29 g, 10.4 mmol, 2.2 equiv.) in THF (15 mL) was added 1 M allylMgBr in ether (9.5 mL, 9.46 mmol, 2.0 equiv.) at 0 °C. After stirring at rt for 1 h, the mixture was cooled to −78 °C. Aldehyde **5** (1.40 g, 4.73 mmol) in THF (15 mL) was added dropwise at −85 °C (dry ice/ether). After stirring at the same temperature for 2 h, the reaction was quenched with MeOH (5 mL), saturated aq. $NaHCO_3$ (7 mL), and 30% H_2O_2 (7 mL). The resulting mixture was stirred at rt for 18 h, and then extracted with EtOAc (50 mL × 3). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, 1% → 3% EtOAc/hexane; ODS, 70% → 80% MeOH) to afford the homoallyl alcohol (1.75 g, 67% ee, which was determined by Mosher analysis as shown in Supplemental Fig. 1). To a solution of the homoallyl alcohol (1.75 g) in THF (13 mL) was added 1 M sodium hexamethyldisilazane (NaHMDS) in THF (6.7 mL, 6.7 mmol) dropwise at 0 °C. After stirring for 30 min, $(Boc)_2O$ (1.47 g, 6.73 mmol) was added, and the reaction mixture was stirred for 15 min at rt. The reaction was quenched with brine (25 mL) and water (10 mL), and the mixture was extracted with EtOAc (50 mL × 3). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, 0.5% → 1% EtOAc/hexane) to afford the carbonate (2.31 g). To a solution of the carbonate (2.31 g) in toluene (25 mL) was added IBr (1.21 g, 5.83 mmol) in CH_2Cl_2 (7.8 mL) at −78 °C. After stirring for 2.5 h at −78 °C, the reaction was quenched with a 1:1 mixture of 10% $Na_2S_2O_3$ and saturated aq. $NaHCO_3$ (80 mL). The mixture was extracted with EtOAc (100 mL × 3). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, 10% → 15% EtOAc/hexane) to afford the iodo carbonate (2.28 g). To a solution of the iodo carbonate (2.28 g) in anhydrous MeOH (25 mL) was added K_2CO_3 (1.84 g, 13.4 mmol). After stirring for 3 h at rt, the reaction was quenched with H_2O (60 mL). The mixture was extracted with

EtOAc (100 mL × 3). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, 10% → 12.5% EtOAc/hexane) to afford **6** (1.40 g, 3.40 mmol, 72% in 4 steps) as a clear oil. ^{1}H NMR: (400 MHz, $CDCl_3$, 0.049 M) δ 0.88 (3H, s), 0.90 (3H, s), 1.24–1.39 (2H, m), 1.52–1.76 (4H, m), 2.42 (1H, dd, J = 4.9, 2.7 Hz), 2.54 (2H, m), 2.73 (1H, dd, J = 4.9, 4.1 Hz), 2.97 (1H, m), 3.79 (3H, s), 4.79 (1H, dd, J = 8.3, 4.5 Hz), 5.03 (2H, s), 6.76–6.82 (3H, m), 7.19 (1H, m), 7.30–7.45 (5H, m) ppm. ^{13}C NMR: (100 MHz, $CDCl_3$, 0.049 M) δ 22.84, 23.05, 25.46, 33.05, 36.65, 37.05, 38.16, 46.33, 50.31, 54.83, 69.91, 82.79, 111.89, 115.12, 121.08, 127.52 (2C), 127.91, 128.56 (2C), 129.28, 137.16, 144.01, 156.06, 158.88 ppm. HR-EIMS: m/z ([M$^+$]): Calcd. for $C_{25}H_{32}O_5$: 412.2250, Found: 412.2272.

Synthesis of **7**. To a solution of **13**[10] (805 mg, 2.14 mmol, 2.0 equiv.) in THF (8.4 mL) was added 1.6 M *n*-BuLi in hexane (1.3 mL, 2.14 mmol, 2.0 equiv.) at rt. After stirring for 10 min at rt, the mixture was cooled to −78 °C. A solution of **6** (439 mg, 1.07 mmol) in THF (4.2 mL) was then added, and the reaction mixture was stirred for 1.5 h at −78 °C. The reaction was quenched with saturated aq. NH_4Cl (10 mL). The mixture was poured into EtOAc (50 mL) and H_2O (30 mL). After the organic layer was separated, the aqueous layer was extracted with EtOAc (50 mL × 2). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to give the complex mixture, which was carried forward without purification. To a solution of the mixture in THF (13 mL) was added $LiAlH_4$ (101 mg, 2.66 mmol) at rt. The resulting mixture was heated at reflux for 2 h, and then the reaction was quenched with 1 N NaOH (10 mL). The mixture was extracted with EtOAc (30 mL × 3). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, 2.5% → 5% → 8% EtOAc/hexane) to afford the diol (550 mg). To a solution of the diol (550 mg) and 2,2-dimethoxypropane (0.92 mL, 7.53 mmol) in CH_2Cl_2 (10 mL) was added (1*R*)-(−)-10-camphorsulfonic acid (CSA, 35 mg, 0.15 mmol) at rt. The mixture was stirred at rt for 45 min, and the reaction was quenched with saturated aq. $NaHCO_4$ (10 mL). The mixture was poured into $CHCl_3$ (10 mL) and the organic layer was separated. The aqueous layer was extracted with $CHCl_3$ (10 mL × 2). The combined organic layers were washed dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was dissolved in THF (8.0 mL), and tetra-*n*-butylammonium fluoride (TBAF, 475 mg, 1.51 mmol) was added at rt. The mixture was stirred at rt for 1 h and then poured into EtOAc (10 mL) and 5% $KHSO_4$ (10 mL), and the organic layer was separated. The aqueous layer was extracted with EtOAc (10 mL × 2). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, 8% → 30% EtOAc/hexane) to afford **7** (454 mg, 0.739 mmol, 69% in 4 steps) as a clear oil. ^{1}H NMR: (500 MHz, $CDCl_3$, 0.007 M) δ 0.82 (3H, s), 0.84 (3H, s), 1.13 (6H, s), 1.18–1.44 (6H, m), 1.33 (3H, s), 1.38 (3H, s), 1.51–1.82 (5H, m), 1.98 (1H, m), 2.22 (1H, dd, J = 16.2, 2.2 Hz), 2.34 (1H, dd, J = 16.2, 5.3 Hz), 2.54 (2H, m), 2.68 (2H, m), 2.84 (1H, m), 3.09 (1H, m), 3.52 (1H, dd, J = 11.4, 2.3 Hz), 3.63 (2H, m), 4.19 (1H, m), 5.05 (2H, s), 6.78–6.82 (3H, m), 7.20 (1H, t, J = 7.8 Hz), 7.31–7.45 (5H, m) ppm. ^{13}C NMR: (125 MHz, $CDCl_3$, 0.007 M) δ 19.52, 22.50, 22.69, 22.78, 22.86, 24.83, 25.69, 26.95, 27.05, 28.30, 30.29, 32.56, 32.98, 36.12, 36.98, 38.48, 43.57, 43.96, 63.54, 63.85, 68.45, 69.98, 75.11, 98.41, 111.75, 115.28, 121.11, 127.52 (2C), 127.92, 128.57 (2C), 129.22, 137.24, 144.66, 158.91 ppm. HR-FABMS: m/z ([M + H]$^+$): Calcd. for $C_{36}H_{55}O_4S_2$: 615.3542, Found: 615.3527.

Synthesis of **8**. To a solution of **7** (454 mg, 0.739 mmol) and NEt_3 (0.52 mL, 3.70 mmol, 5.0 equiv.) in CH_2Cl_2 (8.0 mL) were added DMSO (2.0 mL) and SO_3•pyridine (470 mg, 2.96 mmol, 4.0 equiv.) at rt. The mixture was stirred at rt for 4 h, and the reaction was quenched with saturated aq. NH_4Cl (10 mL). The resulting mixture was poured into EtOAc (50 mL) and saturated aq. NH_4Cl (20 mL). The organic layer was separated and the aqueous layer was extracted with EtOAc (50 mL × 2). The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, 2.5% → 5% EtOAc/hexane) to afford the aldehyde (336 mg). One M $TiCl_4$ in CH_2Cl_2 (78 µL, 78 µmol) was diluted with CH_2Cl_2 (1.25 mL) and cooled to 0 °C. To the $TiCl_4$ solution was added Ti(O*i*-Pr)$_4$ (70 µL, 234 µmol) at 0 °C. The mixture was warmed to rt and stirred for 1 h. Ag_2O (36 mg, 156 µmol) was added in one portion, and stirring was continued with exclusion of direct light for 5 h. (*S*)-1,1′-bi(2-naphthol) (BINOL, 89 mg, 312 µmol) was then added in one portion. After stirring for a further 2 h, the mixture was diluted with CH_2Cl_2 (1.25 mL) to afford the stock solution (about 40 mM) of Ti catalyst. To the aldehyde (320 mg) was added the supernatant (2.6 mL) of the above stock solution at −15 °C. After stirring at the same temperature for 15 min, allyl-$SnBu_3$ (324 µL, 1.05 mmol) was added. The resulting reaction mixture was stirred in a cold room for 20 h. The reaction was quenched with saturated aq. $NaHCO_3$ (10 mL), and poured into EtOAc (30 mL) and H_2O (10 mL). The organic layer was separated, and the aqueous layer was extracted with EtOAc (30 mL × 2). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, 5% → 7.5% EtOAc/hexane) to afford the homo-allyl alcohol (340 mg). To a solution of the homo-allyl alcohol (340 mg) in CH_3CN (9.0 mL) and THF (0.9 mL) were added H_2O (0.9 mL) and TsOH•H_2O (299 mg, 1.57 mmol) at rt. The mixture was stirred at rt for 2 h, and then the reaction was quenched with saturated aq. $NaHCO_3$ (20 mL). The mixture was extracted with EtOAc (30 mL × 3). The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, 10% → 40% EtOAc/hexane) to afford an inseparable mixture of **8** and its diastereomer (274 mg, 0.446 mmol, 63% in 3 steps) as a clear oil. ^{1}H NMR: (400 MHz, $CDCl_3$, 0.049 M) δ 0.85 (3H, s), 0.87 (3H, s), 1.12 (6H, s), 1.24 (1H, m), 1.41–1.69 (9H, m), 1.79 (1H, m), 1.95 (2H, m), 2.12–2.23 (2H, m), 2.33 (1H, m), 2.56 (2H, m), 2.81–3.00 (4H, m), 3.61 (2H, m), 4.47 (1H, m), 5.05 (2H, s), 5.15 (2H, m), 5.82 (1H, m), 6.78–6.84 (3H, m), 7.19 (1H, t, J = 7.8 Hz), 7.30–7.46 (5H, m) ppm. ^{13}C NMR: (100 MHz, $CDCl_3$, 0.049 M) δ 22.46, 22.74, 23.06, 23.32, 25.98, 27.43, 27.64, 32.01, 32.77, 37.14, 37.20, 38.69, 38.76, 42.22, 45.27, 45.74, 45.83, 63.28, 70.05, 71.35, 71.85, 78.39, 111.90, 115.28, 118.51, 121.25, 127.67 (2C), 128.02, 128.68 (2C), 129.34, 134.77, 137.34, 144.83, 158.97 ppm. HR-FABMS: m/z ([M + H]$^+$): Calcd. for $C_{36}H_{55}O_4S_2$: 615.3542, Found: 615.3564.

Synthesis of **9**. To a solution of a mixture of **8** and its diastereomer (255 mg, 0.415 mmol) in MeCN (1.4 mL), CH_2Cl_2 (1.4 mL), and H_2O (1.4 mL) was added $Hg(ClO_4)_2$•$6H_2O$ (422 mg, 0.83 mmol, 2.0 equiv.) at 0 °C. After stirring at the same temperature for 2 h, the reaction mixture was poured into EtOAc (20 mL) and saturated aq. $Na_2S_2O_3$ (20 mL). The organic layer was separated, and the aqueous layer was extracted with EtOAc (20 mL × 2). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (2.5% → 4% → 10% EtOAc/hexane) to afford **9** (88.4 mg, 0.175 mmol, 42%), **10** (45.9 mg, 0.091 mmol, 22%), and **11** (38.1 mg, 0.075 mmol, 18%). Compound **9**: ^{1}H NMR: (400 MHz, $CDCl_3$, 0.079 M) δ 0.82 (3H, s), 0.86 (3H, s), 0.87 (3H, s), 0.96 (3H, s), 1.16 (1H, dd, J = 12.6, 9.0 Hz), 1.36–1.76 (10H, m), 2.24 (3H, m), 2.54 (2H, m), 3.74 (1H, m), 3.78 (1H, d, J = 11.3 Hz, OH), 3.93 (1H, dd, J = 12.4, 2.2 Hz), 4.14 (1H, m), 5.01–5.08 (2H, m), 5.05 (2H, s), 5.79 (1H, m), 6.77–6.85 (3H, m), 7.19 (1H, t, J = 7.8 Hz), 7.30–7.45 (5H, m) ppm. ^{13}C NMR: (100 MHz, $CDCl_3$, 0.079 M) δ 21.80, 23.17, 23.32, 25.46, 25.88, 26.98, 28.27, 31.74, 34.17, 36.16, 37.05, 37.18, 39.47, 41.20, 65.95, 68.40, 69.88, 72.30, 102.59, 111.59, 115.26, 118.17, 121.15, 127.50 (2C), 127.85, 128.53 (2C), 129.14, 134.47, 137.25, 144.84, 158.85 ppm. HR-FABMS: m/z ([M + H]$^+$): Calcd. for $C_{33}H_{47}O_4$: 507.3474, Found: 507.3434. $[\alpha]^{5.6}_D$ +42° (*c* 0.34, $CHCl_3$). Compound **10**: ^{1}H NMR: (400 MHz, $CDCl_3$, 0.079 M) δ 0.84 (3H, s), 0.86 (3H, s), 0.89 (3H, s), 0.97 (3H, s), 1.14 (1H, m), 1.24–1.66 (6H, m), 1.90 (1H, m), 2.16 (2H, m), 1.99–2.19 (4H, m), 2.55 (2H, m), 3.53 (1H, dd, J = 12.4, 1.8 Hz), 3.93 (1H, m), 4.27 (1H, m), 4.96–5.04 (2H, m), 5.05 (2H, s), 5.78 (1H, m), 6.78–6.82 (3H, m), 7.18 (1H, t, J = 7.8 Hz), 7.30–7.45 (5H, m) ppm. ^{13}C NMR: (100 MHz, $CDCl_3$, 0.079 M) δ 22.94, 23.27, 23.34, 25.63, 25.69, 27.71, 32.71, 33.47, 33.82, 36.72, 36.74, 36.97, 38.79, 40.60, 63.09, 68.62, 69.90, 75.60, 101.61, 111.78, 115.15, 115.91, 121.19, 127.51 (2C), 127.89, 128.55

(2C), 129.19, 135.88, 137.20, 144.54, 158.85 ppm. HR-FABMS: m/z ($[M+H]^+$): Calcd. for $C_{33}H_{47}O_4$: 507.3474, Found: 507.3455. $[\alpha]^{6.1}_D$ -15° (c 0.30, $CHCl_3$). Compound **11**: ^{1}H NMR: (400 MHz, $CDCl_3$, 0.079 M) δ 0.87 (3H, s), 0.89 (3H, s), 0.92 (3H, s), 0.96 (3H, s), 1.15 (1H, m), 1.32–1.77 (10H, m), 1.91 (1H, dt, J = 13.3, 4.5 Hz), 2.16 (2H, m), 2.56 (2H, m), 3.51 (1H, dd, J = 12.1, 2.1 Hz), 3.65 (1H, m), 4.09 (1H, m), 4.20 (1H, d, J = 10.2 Hz, OH), 5.05 (2H, s), 5.07–5.12 (2H, m), 5.78 (1H, m), 6.78–6.82 (3H, m), 7.19 (1H, t, J = 7.8 Hz), 7.30–7.45 (5H, m) ppm. ^{13}C NMR: (100 MHz, $CDCl_3$, 0.079 M) δ 22.61, 22.96, 23.48, 25.57, 25.65, 27.62, 31.34, 32.69, 33.58, 36.12, 36.67, 36.98, 38.47, 40.69, 65.23, 69.23, 69.91, 70.06, 102.86, 111.74, 115.20, 117.64, 121.14, 127.51 (2C), 127.90, 128.56 (2C), 129.12, 135.08, 137.20, 144.41, 158.87 ppm. HR-FABMS: m/z ($[M+H]^+$): Calcd. for $C_{33}H_{47}O_4$: 507.3474, Found: 507.3460. $[\alpha]^{5.1}_D$ -17° (c 0.31, $CHCl_3$).

*Synthesis of **12***. To a solution of **14**[10] (31.9 mg, 0.097 mmol, 1.5 equiv.) and Et_3N (15.1 µL, 0.109 mmol, 1.7 equiv.) in toluene (700 µL) was added 2,4,6-trichlorobenzoyl chloride (TCBCl, 17.1 µL, 0.109 mmol, 1.7 equiv.) at rt. After stirring at rt for 2 h, a supernatant of the resulting suspension was added to a solution of **9** (32.6 mg, 0.0644 mmol) and 4-(dimethylamino)pyridine (DMAP, 16.5 mg, 0.135 mmol, 2.1 equiv.) in toluene (700 µL) at rt. The resulting mixture was stirred at 50 °C for 2 h, and then poured into water (10 mL). The mixture was extracted with EtOAc (10 mL × 3). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, 2.5% → 4% → 5% EtOAc/hexane) to afford the ester (34.5 mg). To a vigorously stirred solution of the ester (34.5 mg) in CH_2Cl_2 (2.2 mL) and H_2O (0.4 mL) was added 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ, 19.2 mg, 0.084 mmol) at rt, causing a brown color to appear. After stirring at rt for 1 h, the color gradually changed to yellow-orange with precipitation of a solid. The mixture was poured into saturated aq. $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (10 mL × 3). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, 5% → 30% EtOAc/hexane) to afford the alcohol (26.8 mg). To a solution of the alcohol (26.8 mg) in THF were added imidazole (7.8 mg, 0.115 mmol) and chlorotriethyl silane (TESCl, 7.7 µL, 0.046 mmol) at rt. After stirring at rt for 4 h, the reaction was quenched with brine (5 mL). The resulting mixture was extracted with EtOAc (10 mL × 3). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, 1% EtOAc/hexane) to afford **12** (26.3 mg, 0.0324 mmol, 50% in 3 steps) as a clear oil. ^{1}H NMR: (500 MHz, $CDCl_3$, 0.032 M) δ 0.58 (6H, q, J = 8.0 Hz), 0.78 (3H, s), 0.82 (3H, s), 0.85 (3H, s), 0.92 (9H, t, J = 8.0 Hz), 0.96 (3H, s), 1.08–1.14 (1H, m), 1.29–1.38 (2H, m), 1.40–1.63 (7H, m), 1.73–1.79 (1H, m), 2.17–2.28 (3H, m), 2.46–2.60 (4H, m), 3.35–3.40 (2H, m), 3.45 (1H, dd, J = 9.5, 5.3 Hz), 4.03 (1H, dd, J = 11.3, 3.1 Hz), 4.30–4.35 (1H, m), 4.48–4.53 (2H, m), 4.92–4.99 (2H, m), 5.04 (2H, s), 5.11–5.12 (1H, m), 5.76–5.84 (1H, m), 6.77–6.84 (3H, m), 7.18 (1H, dd, J = 7.8, 7.8 Hz), 7.26–7.44 (10H, m) ppm. ^{13}C NMR: (125 MHz, $CDCl_3$, 0.032 M) δ 4.91 (3C), 6.79 (3C), 21.28, 23.04, 23.05, 25.31, 25.74, 26.41, 26.69, 28.83, 34.64, 36.03, 37.13, 37.21, 39.47, 40.73, 41.11, 68.41, 68.53, 68.64, 69.93, 71.47, 73.33, 74.26, 100.02, 111.66, 115.28, 116.60, 121.21, 127.51 (2C), 127.55, 127.59 (2C), 127.83, 128.31 (2C), 128.52 (2C), 129.10, 134.98, 137.34, 138.26, 144.92, 158.88, 171.58 ppm. HR-FABMS: m/z ($[M+H]^+$): Calcd. for $C_{50}H_{73}O_7Si$: 813.5126, Found: 813.5118. $[\alpha]^{9.3}_D$ $+22^\circ$ (c 1.3, $CHCl_3$).

*Synthesis of **3***. To a suspension of $NaIO_4$ (55.5 mg, 0.259 mmol, 8.0 equiv.) in pH 7.2 phosphate buffer (2.6 mL) was added $KMnO_4$ (5.1 mg, 0.0324 mmol, 1.0 equiv.) in one portion. After stirring at rt for 10 min under an Ar atmosphere, the mixture was added to a solution of **12** (26.3 mg, 0.0324 mmol) in *t*-BuOH (2.6 mL). The reaction mixture was stirred at rt for 1 h, and the reaction was quenched with $Na_2S_2O_3$ (15.4 mg). The resulting mixture was extracted with EtOAc (3 mL × 5). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, 5% → 10% EtOAc/hexane containing 0.5% AcOH) to afford the carboxylic acid (16.1 mg). To a solution of the carboxylic acid (16.1 mg) in THF (1.6 mL) was added a freshly prepared solution of buffered HF•pyridine (75 µL HF•pyridine, 150 µL pyridine, 600 µL THF) at 0 °C. The reaction mixture was stirred for 15 h in a cold room, then diluted with water (2 mL) and warmed to rt. The mixture was extracted with EtOAc (10 mL × 2) and $CHCl_3$ (10 mL × 2). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, 10% → 20% EtOAc/hexane containing 0.5% AcOH) to afford the *seco*-acid (13.6 mg). To a solution of the *seco*-acid (13.6 mg) in toluene (2 mL) were added Et_3N (79 µL, 570 µmol) and TCBCl (55 µL, 380 µmol) at rt. The mixture was stirred at rt for 3 h, and then diluted with toluene (11 mL). The supernatant of the mixture was added dropwise to a solution of DMAP (116 mg, 950 µmol) in toluene (21 mL) over 5 h. The anhydride flask was rinsed twice with toluene (2 mL) (each rinse was added in one portion to the reaction mixture). After stirring at rt for an additional 1 h, saturated aq. $NaHCO_3$ (10 mL) was added, and the resulting biphasic mixture was poured into EtOAc (20 mL). The organic layer was separated, and the aqueous layer was extracted with EtOAc (10 mL × 2). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, 2.5% → 5% → 7.5% EtOAc/hexane) to afford the lactone (10.9 mg). To 10% Pd-C in a double-neck flask was added a solution of the lactone (10.9 mg) in MeOH (0.4 mL) and $CHCl_3$ (0.1 mL) at rt. The mixture was vigorously stirred under a H_2 atmosphere at rt for 5 h. The mixture was filtered, and the filtrate was concentrated *in vacuo*. The residue was purified by HPLC (column A-023; solvent *i*-PrOH: $CHCl_3$: hexane = 5:15:80; flow rate 3.0 mL/min; pressure 500 psi; UV detector 254 nm; retention time 16.2 min) to afford **3** (6.9 mg, 13.4 µmol, 41% in 4 steps) as a clear oil. ^{1}H NMR: (400 MHz, $CDCl_3$, 0.015 M) δ 0.82 (3H, s), 0.88 (3H, s), 0.89 (3H, s), 0.96 (3H, s), 1.14 (1H, dt, J = 12.9, 3.9 Hz), 1.31–1.66 (9H, m), 1.90 (1H, m), 2.41 (1H, dd, J = 13.6, 10.8 Hz), 2.46–2.63 (4H, m), 2.67 (1H, t, J = 5.8 Hz, OH), 2.80 (2H, m), 3.80 (2H, m), 3.94 (1H, m), 4.10 (1H, dd, J = 11.3, 2.6 Hz), 5.15 (1H, m), 5.24 (1H, m), 6.68 (1H, dd, J = 8.0, 2.3 Hz), 6.72 (1H, d, J = 7.6 Hz), 6.76 (1H, s, Ph-OH), 6.95 (1H, br.s), 7.13 (1H, t, J = 7.8 Hz) ppm. ^{13}C NMR: (100 MHz, $CDCl_3$, 0.015 M) δ 21.32, 23.01, 23.34, 23.48, 25.03, 25.96, 27.16, 28.75, 34.33, 35.42, 35.88, 37.13, 37.29, 40.12, 42.83, 64.18, 67.33, 69.13, 70.56, 73.12, 100.65, 112.44, 114.12, 120.58, 129.20, 144.61, 156.58, 169.45, 173.33 ppm. HR-FABMS: m/z ($[M+H]^+$): Calcd. for $C_{29}H_{43}O_8$: 519.2958, Found: 519.2960. $[\alpha]^{25.4}_D$ $+29^\circ$ (c 0.35, $CHCl_3$).

Inhibition of specific binding of [^{3}H]PDBu to PKCδ. The binding of [^{3}H]PDBu to PKCδ was evaluated by the procedure of Sharkey and Blumberg,[25] with 50 mM Tris–maleate buffer (pH 7.4 at 4 °C), 12.5 nM of PKCδ (Invitrogen), 10 nM [^{3}H]PDBu, 50 µg/mL of 1,2-dioleoyl-*sn*-glycero-3-phospho-L-serine (Sigma), 3 mg/mL of bovine γ-globulin (Sigma), and various concentrations of inhibitors. Binding affinity was evaluated based on the concentration required to cause 50% inhibition of the specific binding of [^{3}H]PDBu, IC_{50}, which was calculated with PriProbit 1.63 software. The inhibition constant, K_i, was calculated by the method of Sharkey and Blumberg.[25]

Measurements of cell growth inhibition. The panel of 39 human cancer cell lines established by Yamori[26] and coworkers by the NCI method was employed with modifications, and cell growth inhibition was measured as reported previously.[28] In brief, the cells were plated in 96-well plates in RPMI 1640 medium supplemented with 5% fetal bovine serum, and were allowed to attach overnight. They were incubated with test compounds for 48 h. Cell growth was estimated by sulforhodamine B assay. The 50% growth inhibition (GI_{50}) parameter was calculated as reported previously.[29–31] Absorbance for the control well (C) and the test well (T) was measured at 525 nm along with that for the test well at time 0 (T_0). GI_{50} was calculated as $100 \times [(T - T_0)/(C - T_0)] = 50$.

EBV-EA induction test. Human B-lymphoblastoid Raji cells (5×10^5 cells/mL) were incubated at 37 °C under a 5% CO_2 atmosphere in 1 mL of RPMI 1640 medium (supplemented with 10% fetal bovine serum) with 4 mM sodium *n*-butyrate (a synergist) and 100 nM of test compound in the presence and the absence of 32 nM TPA. TPA was added as 2 µL of a DMSO solution. Since the various

test compounds were added as 2 μL of DMSO solutions of the various 20 μM stock solutions, the final DMSO concentration was 0.4%. After incubation for 48 h, smears were made from the cell suspension, and the EBV-EA-expressing cells were stained by a conventional indirect immunofluorescence technique with an NPC patient's serum (a gift from Kobe University) and FITC-labeled anti-human IgG (Dako, Glostrup, Denmark), as reported previously.[28,29] In each assay, at least 500 cells were counted and the proportion of EA-positive cells was recorded. The test compounds (Aplog-1, bryo-1 from Sigma, and ATX,[32] **3**) did not induce cell death during the experiment at 100 nM. Cell viability exceeded 50% in each experiment.

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