Synthesis and Biological Activities of Acetal Analogs at Position 3 of 10-Methyl-Aplog-1, a Potential Anti-Cancer Lead Derived from Debromoaplysiatoxin

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Dedicated to Prof. Kiyoshi Tomioka on the occasion of his 70th birthday.

ABSTRACT

10-Methyl-Aplog-1 (**1**), a simplified analog of tumor-promoting debromoaplysiatoxin (DAT), is a potential anti-cancer lead because, unlike DAT, **1** is not tumor-promoting or proinflammatory. However, its synthesis required 23 linear steps with an overall yield of 1.1%. To develop a more synthetically accessible compound, we designed a new analog (**2**) whose carbon atom at position 4 was replaced with an oxygen atom. Simultaneous construction of the spiroketal at position 7, the acetal at position 3, and the macrolactone ring enabled us to reduce the synthetic steps to produce **2** and its 3-epimer (**16**) in 18 linear steps with overall yields of 0.30 and 0.67% respectively. Although both analogs retained anti-proliferative activity, it was weaker than that of **1**.

1 INTRODUCTION

Protein kinase C (PKC) is a family of serine/threonine kinases involved in intracellular signal transduction related to proliferation, differentiation, and apoptosis, and serves as a potential target for the treatment of cancer,[1] Alzheimer's disease,[2] and acquired immunodeficiency syndrome (AIDS).[3] Tumor-promoting and highly proinflammatory natural products,[4–6] such as phorbol esters, teleocidins, and aplysiatoxins (Figure 1), also show anti-proliferative and proapoptotic activities towards several cancer cell lines, by binding to conventional (α, βI, βII, γ) and novel (δ, ε, η, θ) PKC isozymes.[7] On the other hand, bryostatin 1 (bryo-1), isolated from marine bryozoan *Bugula neritina*,[8] is a unique PKC activator with little tumor-promoting and proinflammatory activities.[9] Its anti-cancer activity for several solid tumors has thus been investigated in phase I and II clinical trials.[10,11] However, the trials gave rather disappointing results.[12,13] Although bryo-1 antagonized some activities of 12-*O*-tetradecanoylphorbol 13-acetate (TPA),[14] its anti-proliferative activity against several cancer cell lines was not so high.

We focused on natural PKC ligands with anti-proliferative activity as well as tumor-promoting and proinflammatory activities, to develop a new anti-cancer lead by extracting only the anti-proliferative activity related to PKC activation. Recently, we designed Aplog-1, a simplified analog of debromoaplysiatoxin (DAT) isolated from the sea hare *Stylocheilus longicauda*.[6] Aplog-1 suppressed the proliferation of several cancer cell lines but, unlike DAT, did not exhibit tumor-promoting or proinflammatory activities.[15] Subsequent structure-activity studies16 identified 10-methyl-Aplog-1 (**1**) (Figure 2) that had anti-proliferative activity one order of magnitude greater compared with Aplog-1. To our delight, **1** exhibits little tumor-promoting or proinflammatory activities.[16]

Although **1** is a promising anti-cancer lead, its synthesis required 23 linear steps with an overall yield of only 1.1%.[17] To develop a more practical and more synthetically accessible lead, modification of the skeleton was identified as a potential strategy. Kishi's first synthesis of DAT adopted a simultaneous construction of the spiroketal at position 7, the acetal at position 3, and the macrolactone ring,[18] which suggested to us that an acetal analog at position 3 could be more synthetically accessible than the 3-deoxy analogs such as Aplog-1 and 10-methyl-Aplog-1. However, the C-3 hydroxyl group of DAT suffers from dehydration even in chloroform. We therefore designed a new acetal analog of 10-methyl-Aplog-1 (**2**) (Figure 1) whose carbon atom at position 4 was replaced with an oxygen atom. In this paper, the synthesis and biological activities of **2**, as well as its 3-epimer (**16**), are described.

2 RESULTS AND DISCUSSION

2.1 Synthesis of the acetal analogs (2 and 16)

The synthesis of **2** is shown in Scheme 1. A known epoxide **4** was synthesized from *m*-hydroxycinnamic acid (**3**) as reported previously (17% in 10 steps).[17] Then, a hydroxyl group of **4** was protected with a tri-

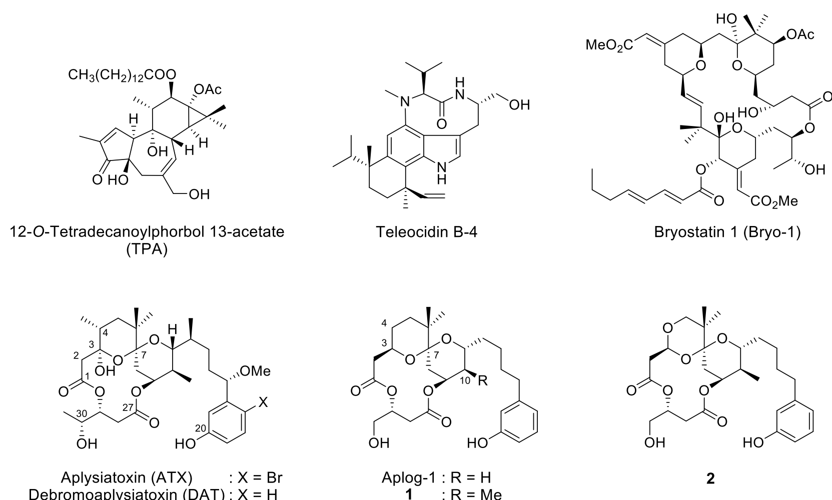
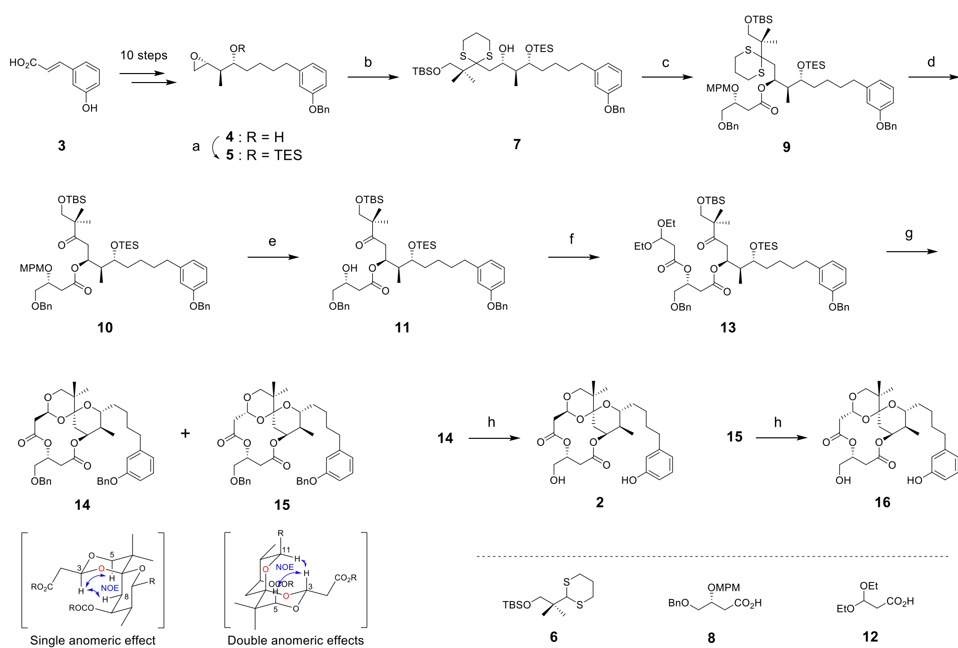


Figure 1: Structures of PKC activators.

ethylsilyl (TES) group. The coupling of an epoxide **5** with a dithiane **6**[19] was achieved by the protocol of Ide and Nakata.[20] Since this step was required strict dry conditions, normal vacuum drying was inappropriate for dehydrating large amounts of compounds.[15] Thus, the small scale reaction was repeated several times. Esterification of **7** with a carboxylic acid **8**[15] followed by deprotection of a thioketal **9** provided **10**. Deprotection of a **4**-methoxyphenylmethyl (MPM) group gave **11**, then Yamaguchi's esterification[21] with a known carboxylic acid **12**[22] provided **13**. For the macrocyclization step, we employed Noyori's condition[23] using trimethylsilyl trifluoromethanesulfonate (TMSOTf) to achieve double cyclic acetalization. This involved removal of the TBS and TES groups and the successive construction of spiroketal at position 7 and cyclic acetal at position 3. This reaction at −50 °C provided **14** and its epimer at position 3 (**15**) in a ratio of approximately 2:3. The relative configurations of **14** and **15** were determined by nuclear Overhauser enhancement (NOE) experiments (Supporting Information). The low yield of this step might be mainly due to the hydrolysis of ester bonds before deprotection of the TBS group. Moreover, **14** was thermodynamically less stable than **15**, which was explained by the anomeric effect at the spiroketal moiety; **15** benefits from double anomeric stabilization while **14** has a single anomeric spiroketal. On the other hand, the ^{1}H-NMR coupling constants of macrolactone ring of **14** were similar to those of dibenzyl-**1** (Supporting Information), suggesting that the replacement of the carbon atom at position 4 with an oxygen atom had no effect on the molecular conformation. Removal of the two benzyl groups of **14** and **15** gave **2** and **16**, respectively (**2**: 0.30%, **16**: 0.67% from **3** in 18 steps).

2.2 Proinflammatory activity of 2 and 16

Our previous studies showed that installing a methyl group at position 4 of **1** significantly increased adverse effects such as tumor-promoting and proinflammatory activities,[24] while a methyl group installed at position 10 or 12 did not enhance these activities.[16,24] Since these undesirable activities seemed to be sensitive to structural modifications around position 4, we first verified the proinflammatory activity of **2** and **16** in the ears of mice. The ear of each ICR mouse was treated with each compound for 24 h. Proinflammatory activity was measured by the increase in the relative weight of each ear after treatment (Figure 2). In



Scheme 1. Synthesis of **2**.

(a) TES-Cl, imidazole, THF, 89%. (b) **6**, *n*-BuLi, THF, 84%. (c) **8**, DCC, DMAP, CH_2Cl_2, 57%. (d) I_2, $NaHCO_3$, MeCN, 63%. (e) DDQ, CH_2Cl_2, H_2O, 83%. (f) **12**, TCB-Cl, Et_3N, DMAP, toluene, 90%. (g) TMSOTf, CH_2Cl_2, **14**: 10%, **15**: 14%. (h) H_2, 10% Pd/C, EtOH, **2**: 78%, **16**: 83%.

contrast to tumor-promoting DAT, **2** and **16** did not show any proinflammatory activity even at 170 nmol. Since proinflammatory activity was well correlated to tumor-promoting activity,[25] **2** and **16** were expected not to promote tumorigenesis.

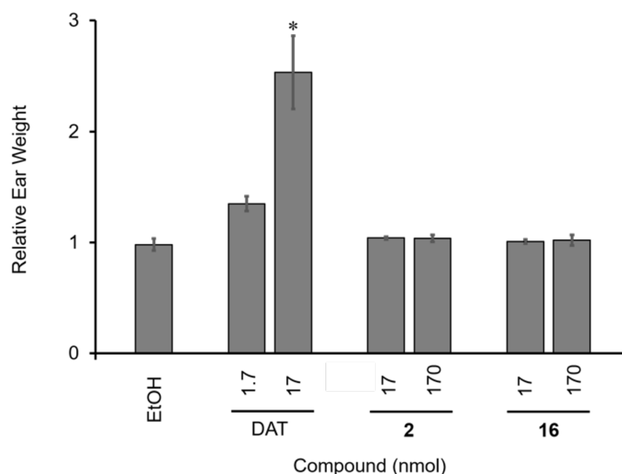


Figure 2: Change in the relative weight of each mouse ear 24 h after the application of debromoaplysiatoxin (DAT), **2**, and **16**. Error bars show the standard deviation of at least two samples. *p < 0.01 vs. vehicle (EtOH) group (Dunnett's test).

2.3 Binding affinity of 2 and 16 for PKC isozymes

PKCα and δ isozymes are predominantly expressed in several human epithelial cancer cell lines, and involved in the cell line-specific anti-proliferative and pro-apoptotic activities of PKC ligands.[26] Thus, we examined the binding affinity of **2** and **16** for PKCα and δ using a competitive binding assay with [^{3}H]phorbol 12,13-dibutyrate (PDBu) as described previously by Sharkey and Blumberg.[27] We used synthetic PKCα-C1A (α-C1A) and PKCδ-C1B (δ-C1B) peptides that are more stable than whole PKCα and δ, produced by molecular biology techniques. These domains are the main binding sites of tumor-promoters, and dissociation constants of several PKC ligands for these peptides were almost equal to those for whole PKC enzymes.[28] As shown in Table 1, the affinities of **2** and **16** for α-C1A and δ-C1B were 5–30 times weaker than that of **1**.[16] As mentioned above, the replacement of the carbon atom at position 4 with an oxygen atom did not alter the molecular conformation. Therefore, the weaker binding affinity of **2** might be attributed to the decreased hydrophobicity around position 4. Comparing **2** and **16**, they showed similar ability to bind to C1 domains of PKC, despite the significant difference in configuration around position 3. Our previous study showed that 20-OH, 27-C=O, and 30-OH groups of aplysiatoxin (ATX) were involved in hydrogen bond with δ-C1B.[29] On the other hand, although 1-C=O of ATX might contribute to generate molecular electrostatic potential complementary to δ-C1B, it was not involved in any hydrogen bond.[29] These observations suggested that the configuration at positions 1-4 of ATX and its analogs was not essential to PKC binding. However, the affinity of **16** for δ-C1B was about two times weaker than that of **2**, while the affinity of **16** for α-C1A was almost the same as that of **2**. These results suggest that δ-C1B recognizes the configuration around position 3 of **2**, while α-C1A did not. Such knowledge might be useful

to develop an isozyme-selective PKC ligand in the future.

Table 1: Values of K_i for the inhibition of [^{3}H]PDBu binding of by **1**, **2**, and **16**

	K_i (nM)		
PKC C1 peptides	**1**[a]	**2**[b]	**16**[b]
α-C1A	4.7	22 (4)	22 (0.9)
δ-C1B	0.46	6.8 (1.3)	13 (0.9)

[a] Cited from Ref. [16]. [b] Values in parentheses represent the standard deviation from at least two separate experiments.

2.4 Anti-proliferative activity of 2 and 16

Finally, we evaluated the anti-proliferative activities of **2** and **16** through growth inhibition tests against 39 human cancer cell lines established by Yamori *et al.*[30] Growth inhibitory activity was expressed as GI_{50} (M), the concentration required to inhibit cell growth by 50% compared to an untreated control. Since **1** showed cell line-selective anti-proliferative activity, the results for the cell lines where **1** showed activity with log GI_{50} values of less than –6.00[16] are listed in Table 2, except for MKN45; the rest of the data are provided in supporting information. The growth inhibitory activities of **2** and **16** against these cell lines were one order of magnitude lower than that of **1**, except for MKN45. Since there was good correlation between the ability to bind to PKC and the anti-proliferative activity against these cell lines,[31] the lower growth inhibitory activity of **2** and **16** could be attributed to their weak binding affinity for PKC.

Table 2: Growth inhibition of **1**, **2**, and **16** against several human cancer cell lines

		log GI_{50} (M)		
Cancer cell line		**1**[a]	**2**	**16**
Breast	HBC-4	−7.48	−6.26	−5.88
	MDA-MB-231	−6.90	−5.10	−4.95
CNS	SNB-78	−6.05	> −5.00	−4.60
Colon	HCC2998	−6.47	−5.56	−5.17
Lung	NCI-H226	−6.15	−5.31	−4.80
	NCI-H460	−7.07	−6.12	−5.69
	A549	−6.01	−5.40	−5.20
Melanoma	LOX-IMVI	−6.21	> −5.00	−4.75
Stomach	St-4	−6.24	−5.96	−5.33
	MKN45	−4.97	−6.00	−5.62

[a] Cited from Ref. [16].

3 CONCLUSION

We synthesized the acetal analog of **1** (**2**) along with its 3-epimer (**16**) in 18 linear steps using the strategy of simultaneously constructing the spiroketal at position 7, the acetal at position 3, and the macrolactone ring, but its overall yield was lower than that of **1** due to the difficulty in the stereocontrol at position 3 and the delayed deprotection of the TBS group. The binding affinity for PKC isozymes and the anti-proliferative activity of **2** were weaker than those of **1**. These results suggest that adequate hydrophobicity

around position 4 is important to develop a promising anti-cancer lead. On the other hand, there was no difference in the binding affinity for PKCα-C1A between **2** and **16**, while the affinity of **16** for PKCδ-C1B was lower than that of **2**. These results suggest that modification of the conformation or configuration at position 3 of **1** may be a useful strategy for designing an isozyme-specific PKC ligand.

4 EXPERIMENTAL

4.1 General remarks

The following spectroscopic and analytical instruments were used: Digital polarimeter, P-2200 (Jasco, Tokyo, Japan); ^{1}H and ^{13}C NMR, AVANCE III 400 and AVANCE III 500 (ref. TMS, Bruker, Germany); HPLC, Model 600E with a Model 2487 UV detector (Waters, Milford, MA, USA); HR-ESI-qTOF-MS, XevoG2-S-qTOF (Waters); HPLC was carried out on a YMC Packed ODS-A (YMC, Kyoto, Japan). Wakogel C-200 (silica gel, Wako Pure Chemical Industries, Osaka, Japan) and YMC A60-350/250 gel (ODS, YMC) were used for column chromatography. [^{3}H]PDBu (18.7 Ci/mmol) was custom-synthesized by PerkinElmer Life Sciences Research Products (Boston, MA, USA). The PKC C1 peptides were synthesized as reported previously.[32] All other chemicals and reagents were purchased from chemical companies and used without further purification. All animal use procedures were approved by Kyoto University Animal Experimentation Committee and performed according to its guidance.

4.2 Synthesis of 2 and 16

(((2*S*,3*R*)-7-(3-(benzyloxy)phenyl)-2-((*R*)-oxiran-2-yl)heptan-3-yl)oxy)triethylsilane (**5**). To a solution of **4** (1.86 g, 5.48 mmol) and imidazole (1.12 g, 16.4 mmol, 3.0 equiv.) in THF (100 mL) was added chlorotriethylsilane (1.38 mL, 8.22 mmol, 1.0 equiv.) at room temperature. The reaction mixture was stirred for 1.5 h at room temperature, and the reaction was quenched with brine (80 mL). The mixture was extracted with EtOAc (100 mL x 3). The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, 2% EtOAc/hexane) to afford **5** (2.22 g, 4.89 mmol, 89%) as a clear oil; $\mathbf{R}_f$: (silica, EtOAc/hexane = 1:9) = 0.51; **IR**: 3034, 2952, 1583, 1487, 1455, 1258, 1155, 1041, 737, 695 cm^{-1}; ^{1}**H NMR**: (500 MHz, $CDCl_3$, 0.043 M): δ 0.58 (6H, q, *J* = 7.9 Hz), 0.91 (3H, d, *J* = 7.1 Hz), 0.95 (9H, d, *J* = 7.9 Hz), 1.33-1.66 (7H, m), 2.43 (1H, dd, *J* = 5.1, 2.8 Hz), 2.59 (2H, t, *J* = 7.4 Hz), 2.70 (1H, dd, *J* = 5.1, 4.2 Hz), 2.92 (1H, m), 3.74 (1H, m), 5.05 (2H, s), 6.78-6.83 (3H, m), 7.19 (1H, t, *J* = 7.8 Hz), 7.30-7.46 (5H, m) ppm; ^{13}**C NMR**: (125 MHz, $CDCl_3$, 0.043 M) : δ 5.2 (3C), 7.0 (3C), 11.9, 25.7, 31.6, 34.0, 36.1, 41.5, 45.2, 53.2, 70.0, 74.6, 111.9, 115.2, 121.2, 127.5 (2C), 127.9, 128.6 (2C), 129.2, 137.3, 144.4, 158.9 ppm; **HR-ESI-qTOF-MS**: *m*/*z* = 477.2823 ([M + Na]$^+$). Calcd. for $C_{28}H_{42}O_3SiNa$ 477.2801; $[\alpha]_D$: +2.40 (*c* = 1.11, $CHCl_3$, 26.0 °C).

(2*S*,3*S*,4*R*)-8-(3-(benzyloxy)phenyl)-1-(2-(1-((tert-butyldimethylsilyl)oxy)-2-methylpropan-2-yl)-1,3-dithian-2-yl)-3-methyl-4-((triethylsilyl)oxy)octan-2-ol (**7**). To a solution of **6** (70 mg, 0.229 mmol, 2.3 equiv.) in THF (0.6 mL) was added 1.6 M *n*-BuLi in hexane (0.142 mL, 0.229 mmol, 2.3 equiv.) at room temperature. After stirring for 1 h at room temperature, the reaction mixture was cooled at 4 °C. A solution of **5** (50 mg, 0.11 mmol) in THF (0.3 mL) was then added, and the reaction mixture was stirred for 3 h at 4 °C. The reaction was quenched with saturated aq. NH_4Cl (1 mL). This procedure was repeated 11 times and a total of 770 mg (2.52 mmol) of **6** was reacted. The combined mixture was extracted with EtOAc (60 mL x 3). The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, 1% → 2% EtOAc/hexane) to afford **7** (774 mg, 1.02 mmol, 84%) as a clear oil; $\mathbf{R}_f$: (silica, EtOAc/hexane = 1:9) = 0.70; **IR**: 3445, 2953, 2857, 1733, 1683, 1583, 1463, 1256, 1155, 1093, 838, 775, 737, 695 cm^{-1}; ^{1}**H NMR**: (500 MHz, $CDCl_3$, 0.022 M): δ 0.05 (6H, s), 0.59 (6H, q, *J* = 7.9 Hz), 0.89 (3H, d, *J* = 7.0 Hz), 0.90 (9H, s), 0.96

(9H, t, J = 7.9 Hz), 1.15 (3H, s), 1.15 (3H, s), 1.30-1.72 (7H, m), 1.88-1.97 (2H, m), 2.10 (1H, dd, J = 15.6, 9.2 Hz), 2.27 (1H, d, J = 15.6 Hz), 2.60 (2H, t, J = 7.6 Hz), 2.77-2.82 (2H, m), 2.89-2.99 (2H, m), 3.72 (2H, s), 4.03 (1H, m), 4.07 (1H, m), 4.20 (1H, s), 5.04 (2H, s), 6.78-6.82 (3H, m), 7.18 (1H, t, J = 7.9 Hz), 7.30-7.44 (5H, m) ppm; **^{13}C NMR**: (125 MHz, $CDCl_3$, 0.022 M): δ −5.4 (2C), 5.3 (3C), 7.0 (3C), 10.7, 18.3, 21.1, 21.2, 23.0, 26.0 (3C), 26.2, 27.2, 27.4, 31.7, 31.7, 36.2, 43.3, 45.3, 48.0, 61.5, 68.2, 70.0, 71.5, 72.6, 111.8, 115.2, 121.2, 127.5 (2C), 127.9, 128.5 (2C), 129.2, 137.3, 144.6, 158.9 ppm; **HR-ESI-qTOF-MS**: m/z = 783.4278 ([M + Na]$^+$). Calcd. for $C_{42}H_{72}O_4S_2Si_2Na$ 784.4308; $[\alpha]_D$: +2.0 (c = 0.71, $CHCl_3$, 25.0 °C).

(2S,3S,4R)-8-(3-(benzyloxy)phenyl)-1-(2-(1-((tert-butyldimethylsilyl)oxy)-2-methylpropan-2-yl)-1,3-dithian-2-yl)-3-methyl-4-((triethylsilyl)oxy)octan-2-yl (R)-4-(benzyloxy)-3-((4-methoxybenzyl)oxy)butanoate (**9**). To **7** (770 mg, 1.01 mmol) were added a solution of **8** (1.07 g, 3.24 mmol, 3.2 equiv.) in CH_2Cl_2 (10 mL), DCC (667 mg, 3.24 mmol, 3.2 equiv.) and DMAP (123 mg, 1.01 mmol, 1.0 equiv.) at room temperature. The reaction mixture was stirred for 18 h at room temperature, and the reaction was quenched with brine (10 mL). To this mixture was added EtOAc (10 mL), and the mixture was extracted with EtOAc (60 mL x 3). The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, 3% → 6% EtOAc/hexane) to afford **9** (615 mg, 0.573 mmol, 57%) as a clear oil and the starting material **7** (236 mg, 0.311 mmol, 31%) was recovered; **R_f**: (silica, EtOAc/hexane = 2:8) = 0.22; **IR**: 2952, 1733, 1717, 1508, 1457, 1249, 1092, 838, 737, 696 cm^{-1}; **^{1}H NMR**: (500 MHz, $CDCl_3$, 0.0063 M): δ 0.05 (6H, s), 0.58 (6H, q, J = 7.9 Hz), 0.88-0.90 (12H, m), 0.94 (9H, t, J = 7.9 Hz), 1.11 (3H, s), 1.12 (3H, s), 1.38-1.66 (5H, m), 1.80-1.85 (4H, m), 2.28 (1H, dd, J = 16.3, 8.7 Hz), 2.47 (1H, d, J = 16.2 Hz), 2.55-2.87 (8H, m), 3.53-3.56 (2H, m), 3.62 (1H, m), 3.69 (1H, d, J = 9.4 Hz), 3.76 (3H, s), 3.79 (1H, m), 4.11 (1H, m), 4.52 (2H, s), 4.54 (1H, d, J = 11.2 Hz), 4.58 (1H, d, J = 11.2 Hz), 5.02 (2H, s), 5.56 (1H, m), 6.77-6.83 (5H, m), 7.15-7.44 (13H, m) ppm; **^{13}C NMR**: (125 MHz, $CDCl_3$, 0.0063 M): δ −5.4, −5.4, 5.2 (3C), 7.1 (3C), 11.5, 14.1, 18.3, 21.5, 23.6, 25.8, 26.0 (3C), 27.7, 27.8, 31.7, 34.0, 36.1, 38.1, 39.4, 42.7, 48.3, 55.3, 60.6, 68.5, 69.9, 72.1 (2C), 73.3, 73.4, 74.4, 74.9, 111.8, 113.7 (2C), 115.1, 121.2, 127.5 (2C), 127.5, 127.6 (2C), 127.9, 128.3 (2C), 128.5 (2C), 129.2, 129.3 (2C), 130.9, 137.3, 138.4, 144.6, 158.9, 159.2, 170.8 ppm; **HR-ESI-qTOF-MS**: m/z = 1095.5598 ([M + Na]$^+$). Calcd. for $C_{61}H_{92}O_8S_2Si_2Na$ 1095.5670; $[\alpha]_D$: +4.3 (c = 0.45, $CHCl_3$, 25.9 °C).

(5R,6S,7S)-5-(4-(3-(benzyloxy)phenyl)butyl)-3,3-diethyl-6,10,10,13,13,14,14-heptamethyl-9-oxo-4,12-dioxa-3,13-disilapentadecan-7-yl (R)-4-(benzyloxy)-3-((4-methoxybenzyl)oxy)butanoate (**10**). To a solution of **9** (433 mg, 0.404 mmol) in MeCN (55 mL) were added saturated aq. $NaHCO_3$ (18.5 mL) and iodine (410 mg, 1.62 mmol, 4.0 equiv.) in MeCN (35 mL) at 4 °C. After stirring for 2 h at 4 °C, the reaction mixture was quenched with saturated aq. $NaHCO_3$ (50 mL) and 20% Na_2SO_4 (50 mL). The mixture was stirred for 10 min and extracted with EtOAc (80 mL x 3). The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, 5% EtOAc/hexane) to afford **10** (250 mg, 0.255 mmol, 63%) as a clear oil; **R_f**: (silica, EtOAc/hexane = 2:8) = 0.51; **IR**: 2954, 2876, 1733, 1612, 1584, 1514, 1456, 1250, 1173, 1102, 839, 777, 738, 696 cm^{-1}; **^{1}H NMR**: (500 MHz, $CDCl_3$, 0.032 M): δ 0.02 (3H, s), 0.02 (3H, s), 0.56 (6H, q, J = 8.0 Hz), 0.85-0.89 (12H, m), 0.94 (9H, t, J = 8.0 Hz), 1.04 (3H, s), 1.06 (3H, s), 1.37-1.42 (3H, m), 1.58-1.63 (3H, m), 1.96 (1H, m), 2.51 (2H, d, J = 6.5 Hz), 2.54-2.59 (2H, m), 2.82-2.91 (2H, m), 3.50 (2H, d, J = 5.0 Hz), 3.53 (2H, s), 3.60 (1H, m), 3.76 (3H, s), 4.08 (1H, m), 4.52 (2H, s), 4.53 (1H, d, J = 11.2 Hz), 4.56 (1H, d, J = 11.2 Hz), 5.03 (2H, s), 5.48 (1H, m), 6.76-6.84 (5H, m), 7.15-7.44 (13H, m) ppm; **^{13}C NMR**: (125 MHz, $CDCl_3$, 0.032 M) : δ −5.6, −5.6, 5.2 (3C), 7.0 (3C), 11.2, 18.2, 21.4, 21.5, 25.5, 25.9 (3C), 31.6, 33.8, 36.1, 37.7, 39.8, 40.8, 49.5, 55.3, 69.8, 69.9, 70.6, 72.0, 72.2, 73.3, 74.1, 74.9, 112.0, 113.7 (2C), 115.1, 121.2, 127.5 (3C), 127.6 (2C), 127.8, 128.3 (2C), 128.5 (2C), 129.2, 129.4 (2C), 130.9, 137.3, 138.4, 144.5, 158.9, 159.2, 170.5, 211.5 ppm; **HR-ESI-qTOF-MS**: m/z = 1005.5712 ([M + Na]$^+$). Calcd. for $C_{58}H_{86}O_9Si_2Na$ 1005.5708.; $[\alpha]_D$: −0.40 (c = 0.74, $CHCl_3$, 25.6 °C).

(5R,6S,7S)-5-(4-(3-(benzyloxy)phenyl)butyl)-3,3-diethyl-6,10,10,13,13,14,14-heptamethyl-9-oxo-4,12-dioxa-3,13-disilapentadecan-7-yl (R)-4-(benzyloxy)-3-hydroxybutanoate (**11**). To a mixture of **10**

(250 mg, 254 mmol), in CH_2Cl_2 (31 mL) and pH 7.2 phosphate buffer (6.2 mL, 319252, Fluka^{TM}) was added DDQ (115 mg, 0.508 mmol, 2.0 equiv.) at 4 ºC. After stirring for 3 h at 4 ºC, the reaction was quenched with saturated aq. $NaHCO_3$ (80 mL). The mixture was extracted with EtOAc (80 mL x 3). The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, 7% EtOAc/hexane) to afford **11** (182 mg, 0.181 mmol, 83%) as a clear oil; **R_f**: (silica, EtOAc/hexane = 2:8) = 0.35; **IR**: 3525, 3033, 2931, 1733, 1601, 1583, 1456, 1382, 1257, 1156, 1103, 839, 777, 737, 696 cm^{-1}; **^{1}H NMR**: (500 MHz, $CDCl_3$, 0.037 M): δ 0.02 (3H, s), 0.03 (3H, s), 0.57 (6H, q, *J* = 8.0 Hz), 0.87 (9H, s), 0.89 (3H, d, *J* = 7.1 Hz), 0.94 (9H, t, *J* = 8.0 Hz), 1.06 (3H, s), 1.08 (3H, s), 1.36-1.43 (3H, m), 1.52-1.64 (3H, m), 1.97 (1H, m), 2.43 (1H, dd, *J* = 16.0, 7.9 Hz), 2.47 (1H, dd, *J* = 16.0, 4.9 Hz), 2.57-2.61 (2H, m), 2.83 (1H, dd, *J* = 18.1, 9.7 Hz), 2.95 (1H, dd, *J* = 18.1, 2.5 Hz), 3.14 (1H, m), 3.44-3.49 (2H, m), 3.54 (2H, s), 3.60 (1H, m), 4.20 (1H, m), 4.54 (1H, d, *J* = 12.2 Hz), 4.56 (1H, d, *J* = 12.2 Hz), 5.05 (2H, s), 5.45 (1H, ddd, *J* = 9.5, 5.8, 2.5 Hz), 6.77-6.83 (3H, m), 7.18 (1H, t, *J* = 7.9 Hz), 7.27-7.45 (10H, m) ppm; **^{13}C NMR**: (125 MHz, $CDCl_3$, 0.037 M): δ −5.6, −5.6, 5.2 (3C), 7.0 (3C), 11.1, 18.2, 21.4, 21.5, 25.5, 25.8 (3C), 31.5, 33.7, 36.0, 38.8, 39.7, 40.8, 49.6, 67.2, 70.0 (2C), 70.7, 73.3, 73.4, 74.0, 112.0, 115.1, 121.2, 127.5 (2C), 127.7 (3C), 127.8, 128.4 (2C), 128.5 (2C), 129.2, 137.3, 138.1, 144.5, 158.9, 171.1, 212.4 ppm; **HR-ESI-qTOF-MS**: *m/z* = 885.5137 ([M + Na]$^+$). Calcd. for $C_{50}H_{78}O_8Si_2Na$ 885.5133; $[\alpha]_D$: +2.8 (*c* = 0.55, $CHCl_3$, 25.8 °C).

(5*R*,6*S*,7*S*)-5-(4-(3-(benzyloxy)phenyl)butyl)-3,3-diethyl-6,10,10,13,13,14,14-heptamethyl-9-oxo-4,12-dioxa-3,13-disilapentadecan-7-yl (*R*)-4-(benzyloxy)-3-((3,3-diethoxypropanoyl)oxy)butanoate (**13**). To a solution of **12** (58.6 mg, 0.362 mmol, 1.5 equiv.) in toluene (10 mL) were added Et_3N (55.1 μL, 0.398 mmol, 1.9 equiv.) and TCB-Cl (56.7 μL, 0.362 mmol, 1.5 equiv.) at room temperature. After stirring for 2 h at room temperature, the resulting suspension was added to a solution of **11** (182 mg, 0.211 mmol) and DMAP (48.6 mg, 0.398 mmol, 1.9 equiv.) in toluene (5 mL) at room temperature. The reaction mixture was stirred for 2.5 h at room temperature and quenched with saturated aq. $NaHCO_3$ (100 mL). The mixture was extracted with EtOAc (80 mL x 3). The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, 5% EtOAc/hexane) to afford **13** (191 mg, 0.190 mmol, 90%) as a clear oil; **R_f**: (silica, EtOAc/hexane = 2:8) = 0.46; **IR**: 2954, 2932, 1741, 1457, 1376, 1257, 1104, 838, 776, 741, 697 cm^{-1}; **^{1}H NMR**: (500 MHz, $CDCl_3$, 0.029 M): δ 0.02 (3H, s), 0.02 (3H, s), 0.56 (6H, q, *J* = 7.9 Hz), 0.80-0.88 (12H, m), 0.94 (9H, t, *J* = 7.9 Hz), 1.05 (3H, s), 1.06 (3H, s), 1.16 (6H, t, *J* = 7.1 Hz), 1.35-1.43 (3H, m), 1.54-1.63 (3H, m), 1.94 (1H, m), 2.53-2.71 (6H, m), 2.84 (1H, dd, *J* = 18.2, 8.7 Hz), 2.89 (1H, dd, *J* = 18.2, 3.5 Hz), 3.48-3.55 (4H, m), 3.57-3.66 (5H, m), 4.50 (1H, d, *J* = 12.0 Hz), 4.55 (1H, d, *J* = 12.0 Hz), 4.93 (1H, t, *J* = 5.9 Hz), 5.05 (2H, s), 5.34 (1H, m), 5.45 (1H, m), 6.77-6.83 (3H, m), 7.18 (1H, t, *J* = 7.8 Hz), 7.27-7.45 (10H, m) ppm; **^{13}C NMR**: (125 MHz, $CDCl_3$, 0.029 M): δ −5.6, −5.6, 5.2 (3C), 7.0 (3C), 11.2, 15.3 (2C), 18.2, 21.4, 21.5, 25.5, 25.8 (3C), 31.6, 33.9, 36.0, 36.0, 39.6, 40.1, 40.7, 49.4, 61.8, 62.0, 69.7, 69.8, 69.9, 70.4, 70.8, 73.3, 74.1, 99.7, 112.0, 115.1, 121.2, 127.5 (2C), 127.6 (3C), 127.8, 128.4 (2C), 128.5 (2C), 129.2, 137.4, 138.1, 144.5, 158.9, 169.1, 169.2, 211.4 ppm; **HR-ESI-qTOF-MS**: *m/z* = 1029.5874 ([M + Na]$^+$). Calcd. for $C_{57}H_{90}O_{11}Si_2Na$ 1029.5919; $[\alpha]_D$: +2.0 (*c* = 0.71, $CHCl_3$, 21.8 °C).

(1*S*,3*R*,4*R*,5*S*,9*R*,13*S*)-9-((benzyloxy)methyl)-3-(4-(3-(benzyloxy)phenyl)butyl)-4,16,16-trimethyl-2,6,10,14,17-pentaoxatricyclo[11.3.1.11,5]octadecane-7,11-dione (**14**) and (1*S*,3*R*,4*R*,5*S*,9*R*,13*R*)-9-((benzyloxy)methyl)-3-(4-(3-(benzyloxy)phenyl)butyl)-4,16,16-trimethyl-2,6,10,14,17-pentaoxatricyclo[11.3.1.11,5]octadecane-7,11-dione (**15**). To a solution of **13** (16.9 mg, 0.0168 mmol) in CH_2Cl_2 (0.75 mL) was added TMSOTf (3.0 μL, 0.0168 mmol, 1.0 equiv.) in CH_2Cl_2 (0.1 mL) at −50 ºC. The reaction mixture was stirred for 5 min at −50 ºC, and the reaction was quenched with saturated aq. $NaHCO_3$ (1 mL) at 4 ºC. The mixture was extracted with EtOAc (2 mL x 3). The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, 8% EtOAc/hexane) and purified by HPLC (column, YMC Packed ODS-A AA12S05-2510WT; solvent, MeOH/H_2O = 92.5%, flow rate 3.0 mL/min; UV detector 254 nm) to afford **14** (1.2 mg, 1.8 μmol, 10%), and **15** (1.6 mg, 2.3 μmol, 14%); **Data for 14**: **R_f**: (silica, EtOAc/hexane = 2:8) = 0.39; **IR**: 2931, 2859, 1729, 1582,

1456, 1261, 1120, 739, 697 cm^{-1}; **^{1}H NMR**: (500 MHz, $CDCl_3$, 0.0034 M): δ 0.75 (3H, s), 0.79 (3H, d, *J* = 6.9 Hz), 1.12 (3H, s), 1.30-1.71 (8H, m), 2.57-2.62 (4H, m), 2.75 (1H, dd, *J* = 12.9, 3.1 Hz), 2.80 (1H, dd, *J* = 16.7, 3.9 Hz), 2.87 (1H, dd, *J* = 16.7, 10.2 Hz), 3.40 (1H, d, *J* = 11.6 Hz), 3.53 (1H, d, *J* = 11.6 Hz), 3.59 (1H, dd, *J* = 10.1, 5.6 Hz), 3.67 (1H, dd, *J* = 10.1, 4.0 Hz), 3.92 (1H, m), 4.50 (1H, d, *J* = 12.0 Hz), 4.56 (1H, d, *J* = 12.0 Hz), 5.00 (1H, dd, *J* = 9.0, 3.1 Hz), 5.04 (1H, m), 5.05 (2H, s), 5.14 (1H, m), 6.78-6.85 (3H, m), 7.19 (1H, t, *J* = 7.9 Hz), 7.26-7.45 (10H, m) ppm; **^{13}C NMR**: (125 MHz, $CDCl_3$, 0.0034 M): δ 13.1, 20.2, 21.0, 24.3, 26.9, 31.1, 32.3, 36.1, 36.8, 37.0, 37.7, 41.5, 68.8, 69.5, 70.0, 70.0, 72.1, 73.5, 74.4, 94.6, 99.7, 111.9, 115.2, 121.3, 127.5 (2C), 127.7 (2C), 127.8, 127.9, 128.5 (2C), 128.5 (2C), 129.2, 137.4, 137.7, 144.7, 158.9, 168.0, 170.0 ppm; **HR-ESI-qTOF-MS**: *m/z* = 709.3372 ([M + Na]$^+$). Calcd. for $C_{41}H_{50}O_9Na$ 709.3353; $[\alpha]_D$: +33 (*c* = 0.080, $CHCl_3$, 28.5 °C); **Data for 15**: **R_f**: (silica, EtOAc/hexane = 2:8) = 0.42; **IR**: 2935, 1733, 1717, 1582, 1457, 1388, 1308, 1177, 1154, 1057, 1011, 970, 752, 697 cm^{-1}; **^{1}H NMR**: (500 MHz, $CDCl_3$, 0.0076 M): δ 0.73 (3H, s), 0.85 (3H, d, *J* = 6.9 Hz), 1.09 (3H, s), 1.36-1.73 (8H, m), 2.23 (1H, dd, *J* = 14.7, 2.7 Hz), 2.54-2.64 (4H, m), 2.75 (1H, dd, *J* = 17.9, 2.2 Hz), 2.88 (1H, dd, *J* = 17.9, 12.8 Hz), 3.24 (1H, d, *J* = 10.9 Hz), 3.45-3.50 (2H, m), 3.56 (1H, m), 3.87 (1H, d, *J* = 10.9 Hz), 4.49 (1H, d, *J* = 12.0 Hz), 4.56 (1H, d, *J* = 12.0 Hz), 4.79 (1H, m), 5.04-5.06 (3H, m), 5.38 (1H, m), 6.79-6.86 (3H, m), 7.19 (1H, t, *J* = 7.9 Hz), 7.27-7.44 (10H, m) ppm; **^{13}C NMR**: (125 MHz, $CDCl_3$, 0.0076 M): δ 13.9, 19.6, 22.0, 24.9, 31.0, 31.4, 32.6, 36.1, 36.4, 37.0, 37.4, 41.0, 69.3, 69.4, 70.0, 70.4, 73.4, 73.5, 74.3, 91.7, 99.4, 111.9, 115.3, 121.2, 127.5 (2C), 127.7 (2C), 127.8, 127.9, 128.5 (2C), 128.6 (2C), 129.2, 137.3, 137.8, 144.5, 159.0, 169.5, 169.8 ppm; **HR-ESI-qTOF-MS**: *m/z* = 709.3372 ([M + Na]$^+$). Calcd. for $C_{41}H_{50}O_9Na$ 709.3353; $[\alpha]_D$: +100 (*c* = 0.22, $CHCl_3$, 17.9 °C).

(1*S*,3*R*,4*R*,5*S*,9*R*,13*S*)-9-(hydroxymethyl)-3-(4-(3-hydroxyphenyl)butyl)-4,16,16-trimethyl-2,6,10,14,17-pentaoxatricyclo[11.3.1.11,5]octadecane-7,11-dione (**2**). To a solution of **14** (0.7 mg, 1.0 μmol) in EtOH (100 μL) was added 10% Pd-C (0.5 mg) at room temperature. The reaction mixture was stirred for 2 h at room temperature under H_2. The mixture was filtered, and purified by HPLC (column, YMC Packed ODS-A AA12S05-2510WT; solvent, MeOH/H_2O = 92.5%, flow rate 3.0 mL/min; UV detector 254 nm) to afford **2** (0.4 mg, 0.79 μmol, 79%); **R_f**: (silica, EtOAc/hexane = 1:1) = 0.23; **IR**: 3422, 2938, 1725, 1588, 1458, 1391, 1302, 1155, 1121, 1029, 994, 969, 756, 696 cm^{-1}; **^{1}H NMR**: (400 MHz, $CDCl_3$, 0.0020 M): δ 0.77 (3H, s), 0.79 (3H, d, *J* = 6.9 Hz), 1.11 (3H, s), 1.33-1.62 (6H, m), 1.72 (1H, dd, *J* = 15.5, 3.9 Hz), 1.77 (1H, m), 2.22 (1H, t, *J* = 6.0 Hz), 2.52-2.59 (2H, m), 2.64-2.71 (2H, m), 2.81-2.87 (3H, m), 3.41 (1H, d, *J* = 11.6 Hz), 3.55 (1H, d, *J* = 11.6 Hz), 3.81-3.84 (2H, m), 4.00 (1H, m), 5.03 (1H, dd, *J* = 6.4, 3.3 Hz), 5.06 (1H, dd, *J* = 9.1, 3.2 Hz), 5.13 (1H, m), 6.10 (1H, s), 6.68 (1H, dd, *J* = 8.1, 2.6 Hz), 6.73 (1H, d, *J* = 7.5 Hz), 6.82 (1H, m), 7.13 (1H, t, *J* = 7.7 Hz) ppm; **^{13}C NMR**: (125 MHz, $CDCl_3$, 0.0026 M) : δ 13.1, 20.2, 21.0, 24.0, 26.9, 29.3, 31.4, 35.4, 36.6, 37.4, 37.9, 41.6, 63.9, 67.6, 72.5, 72.8, 74.4, 94.6, 99.9, 112.7, 114.9, 120.7, 129.4, 144.7, 156.2, 169.6, 170.2 ppm; **HR-ESI-qTOF-MS**: *m/z* = 529.2417 ([M + Na]$^+$). Calcd. for $C_{27}H_{38}O_9Na$ 529.2414; $[\alpha]_D$: +131 (*c* = 0.050, $CHCl_3$, 28.8 °C).

(1*S*,3*R*,4*R*,5*S*,9*R*,13*R*)-9-(hydroxymethyl)-3-(4-(3-hydroxyphenyl)butyl)-4,16,16-trimethyl-2,6,10,14,17-pentaoxatricyclo[11.3.1.11,5]octadecane-7,11-dione (**16**). To a solution of **15** (4.7 mg, 6.9 μmol) in EtOH (250 μL) was added 10% Pd-C (2.0 mg) at room temperature. The reaction mixture was stirred for 2 h at room temperature under H_2. The mixture was filtered, and purified by HPLC (column, YMC Packed ODS-A AA12S05-2510WT; solvent, MeOH/H_2O = 92.5%, flow rate 3.0 mL/min; UV detector 254 nm) to afford **16** (2.9 mg, 5.7 μmol, 83%); **R_f**: (silica, EtOAc/hexane = 1:1) = 0.26; **IR**: 3423, 2936, 1726, 1588, 1459, 1389, 1310, 1179, 1131, 1055, 1010, 970, 754, 696 cm^{-1}; **^{1}H NMR**: (500 MHz, $CDCl_3$, 0.0040 M): δ 0.73 (3H, s), 0.84 (3H, d, *J* = 6.9Hz), 1.10 (3H, s), 1.35-1.70 (8H, m), 1.97 (1H, t, *J* = 6.0 Hz), 2.26 (1H, dd, *J* = 14.8, 2.7 Hz), 2.60-2.67 (4H, m), 2.70 (1H, dd, *J* = 17.8, 2.0 Hz), 2.87 (1H, dd, *J* = 17.8, 11.9 Hz), 3.25 (1H, d, *J* = 10.9 Hz), 3.54 (1H, m), 3.69-3.76 (2H, m), 3.86 (1H, d, *J* = 10.9 Hz), 4.79 (1H, m), 5.07 (1H, dd, *J* = 9.2, 3.8 Hz), 5.35 (1H, m), 5.43 (1H, s), 6.67 (1H, dd, *J* = 7.9, 2.0 Hz), 6.75-6.77 (2H, m), 7.15 (1H, t, *J* = 7.7 Hz) ppm; **^{13}C NMR**: (125 MHz, $CDCl_3$, 0.017 M) : δ 13.9, 19.6, 22.0, 24.6, 30.7, 31.0, 32.4, 35.6, 36.5, 36.8, 37.3, 41.0, 64.3, 69.4, 72.1, 73.5, 74.2, 91.6, 99.4, 112.8, 115.6, 121.0, 129.5, 144.5, 155.7, 169.0, 171.2 ppm; **HR-ESI-qTOF-MS**: *m/z* = 529.2417 ([M + Na]$^+$). Calcd. for $C_{27}H_{38}O_9Na$ 529.2414;

$[\alpha]_D$: +79 (c = 0.10, $CHCl_3$, 28.6 °C).

4.3 Mouse ear swelling test.

This test was performed as reported previously.[33] A solution of each test compound in EtOH (10 μL) or EtOH (vehicle) was applied to the right ear of ICR mice (Shimizu Laboratory Supplies, Japan) by means of micropipette. A volume of 5 μL was delivered to both the inner and outer surfaces of the ear. After 24 h, a disk (0.8-cm square) was obtained from the ear and weighed. Each group consisted of at least two mice.

4.4 Measurements of cell growth inhibition.

A panel of 39 human cancer cell lines established by Yamori and colleagues[30] according to the NCI method with modifications was employed, and cell growth inhibitory activity was measured as reported previously. In brief, the cells were plated in 96-well plates in RPMI 1640 medium supplemented with 5% fetal bovine serum and allowed to attach overnight. The cells were incubated with each test compound for 48 h. Cell growth was estimated by the sulforhodamine B assay. The 50% growth inhibition (GI_{50}) parameter was calculated as reported previously.[34] 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). Cell growth inhibition (% growth) by each concentration of drug (**2**: 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, and 10^{-5} M; **16**: 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, and 10^{-4} M) was calculated as $100[(T - T_0)/(C - T_0)]$ using the average of duplicate points. By processing these values, each GI_{50} value, defined as $100[(T - T_0)/(C - T_0)] = 50$, was determined.

4.5 Inhibition of specific binding of $[^3H]$PDBu to the PKC C1 peptides.

The binding of $[^3H]$PDBu to the PKCα-C1A and δ-C1B peptides was evaluated by the procedure of Sharkey and Blumberg[27] with modifications as reported previously[28] with 50 mM Tris-maleate buffer (pH 7.4 at 4 ºC), 40 nM α-C1A peptide or 13.8 nM δ-C1B peptide, 20 nM $[^3H]$PDBu (18.7 Ci/mmol), 50 μg/mL 1,2-dioleoyl-*sn*-glycero-3-phospho-L-serine (Sigma), 3 mg/mL bovine γ-globulin (Sigma), and various concentrations of an inhibitors. Binding affinity was evaluated based on the concentration required to cause 50% inhibition of the specific binding of $[^3H]$PDBu, IC_{50}, which was calculated by log-probit regression analysis. The inhibition constant, K_i, was calculated by the method of Sharkey and Blumberg.[27]

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References

[1] Michael Fahrmann. Targeting protein kinase C (PKC) inphysiology and cancer of the gastric cell system. *Current Medicinal Chemistry*, 15(12):1175–1191, may 2008.

[2] Mary J. Savage, Stephen P. Trusko, David S. Howland, Leonard R. Pinsker, Suzanne Mistretta, Andrew G. Reaume, Barry D. Greenberg, Robert Siman, and Richard W. Scott. Turnover of Amyloid β-Protein in Mouse Brain and Acute Reduction of Its Level by Phorbol Ester. *The Journal of Neuroscience*, 18(5):1743–1752, mar 1998.

[3] Kenneth A. Roebuck, De Sheng Gu, and Martin F. Kagnoff. Activating protein-1 cooperates with phorbol ester activation signals to increase HIV-1 expression. *AIDS (London, England)*, 10(8):819–26, jul 1996.

[4] S. Ohno and Y. Nishizuka. Protein kinase C isotypes and their specific functions: Prologue. *Journal of Biochemistry*, 132(4):509–511, oct 2002.

[5] Hirota Fujiki and Takashi Sugimura. New Classes of Tumor Promoters: Teleocidin, Aplysiatoxin, and Palytoxin. *Advances in Cancer Research*, 49:223–264, 1987.

[6] Yoshinori Kato and Paul J. Scheuer. Aplysiatoxin and debromoaplysiatoxin, constituents of the marine mollusk *Stylocheilus longicauda*. *Journal of the American Chemical Society*, 96(7):2245–2246, apr 1974.

[7] Alexandra C. Newton. Protein kinase C: Structural and spatial regulation by phosphorylation, cofactors, and macromolecular interactions. *Chemical Reviews*, 101(8):2353–2364, 2001.

[8] George R. Pettit, Cherry L. Herald, Dennis L. Doubek, Delbert L. Herald, Edward Arnold, and Jon Clardy. Isolation and structure of bryostatin 1. *Journal of the American Chemical Society*, 104(24):6846–6848, dec 1982.

[9] M G Kazanietz, N E Lewin, F Gao, G R Pettit, and P M Blumberg. Binding of [26-^{3}H]bryostatin 1 and analogs to calcium-dependent and calcium-independent protein kinase C isozymes. *Molecular pharmacology*, 46(2):374–9, aug 1994.

[10] Patricia S Lorenzo and Phillip A Dennis. Modulating protein kinase C (PKC) to increase the efficacy of chemotherapy: stepping into darkness. *Drug Resistance Updates*, 6(6):329–339, dec 2003.

[11] Peter Kollár, Josef Rajchard, Zuzana Balounová, and Jiří Pazourek. Marine natural products: Bryostatins in preclinical and clinical studies. *Pharmaceutical Biology*, 52(2):237–242, 2014.

[12] B.-F. Ruan and H.-L. Zhu. The Chemistry and Biology of the Bryostatins: Potential PKC Inhibitors in Clinical Development. *Current Medicinal Chemistry*, 19(16):2652–2664, apr 2012.

[13] Ying-Qing Wang and Ze-Hong Miao. Marine-Derived Angiogenesis Inhibitors for Cancer Therapy. *Marine Drugs*, 11(12):903–933, mar 2013.

[14] Henry Hennings, Peter M. Blumberg, George R. Pettit, Cherry L. Herald, Robert Shores, and Stuart H. Yuspa. Bryostatin 1, an activator of protein kinase C, inhibits tumor promotion by phorbol esters in SENCAR mouse skin. *Carcinogenesis*, 8(9):1343–1346, 1987.

[15] Yu Nakagawa, Ryo C. Yanagita, Naoko Hamada, Akira Murakami, Hideyuki Takahashi, Naoaki Saito, Hiroshi Nagai, and Kazuhiro Irie. A simple analogue of tumor-promoting aplysiatoxin is an antineoplastic agent rather than a tumor promoter: Development of a synthetically accessible protein kinase C activator with bryostatin-like activity. *Journal of the American Chemical Society*, 131(22):7573–7579, 2009.

[16] Masayuki Kikumori, Ryo C. Yanagita, Harukuni Tokuda, Nobutaka Suzuki, Hiroshi Nagai, Kiyotake Suenaga, and Kazuhiro Irie. Structure-activity studies on the spiroketal moiety of a simplified analogue of debromoaplysiatoxin with antiproliferative activity. *Journal of Medicinal Chemistry*, 55(11):5614–5626, 2012.

[17] Masayuki Kikumori, Ryo C. Yanagita, and Kazuhiro Irie. Improved and large-scale synthesis of 10-methyl-aplog-1, a potential lead for an anticancer drug. *Tetrahedron*, 70(52):9776–9782, 2014.

[18] Pyeong Uk Park, Chris a. Broka, Bruce F. Johnson, and Yoshito Kishi. Total synthesis of debromoaplysiatoxin and aplysiatoxin. *Journal of the American Chemical Society*, 109(20):6205–6207, sep 1987.

[19] Christoph W. Wullschleger, Jürg Gertsch, and Karl-Heinz Altmann. Stereoselective Synthesis of a Monocyclic Peloruside A Analogue. *Organic Letters*, 12(5):1120–1123, mar 2010.

[20] Mitsuaki Ide and Masaya Nakata. Room-Temperature Metallation of 2-Substituted 1,3-Dithiane Derivatives and Subsequent Coupling with 2,3-Disubstituted Oxiranes. *Bulletin of the Chemical Society of Japan*, 72(11):2491–2499, nov 1999.

[21] Yasuhiro Kawanami, Yuichiro Dainobu, Junji Inanaga, Tsutomu Katsuki, and Masaru Yamaguchi. Synthesis of Thiol Esters by Carboxylic Trichlorobenzoic Anhydrides, 1981.

[22] Florencio Zaragoza, Henrik Stephensen, Bernd Peschke, and Karin Rimvall. 2-(4-Alkylpiperazin-1-yl)quinolines as a New Class of Imidazole-Free Histamine H_3 Receptor Antagonists. *Journal of Medicinal Chemistry*, 48(1):306–311, jan 2005.

[23] T. Tsunoda, M. Suzuki, and R. Noyori. A facile procedure for acetalization under aprotic conditions. *Tetrahedron Letters*, 21(14):1357–1358, jan 1980.

[24] Masayuki Kikumori, Ryo C. Yanagita, Harukuni Tokuda, Kiyotake Suenaga, Hiroshi Nagai, and Kazuhiro Irie. Structural optimization of 10-methyl-aplog-1, a simplified analog of debromoaplysiatoxin, as an anticancer lead. *Bioscience, Biotechnology, and Biochemistry*, 80(2):221–231, 2016.

[25] Mary Philip, Donald A. Rowley, and Hans Schreiber. Inflammation as a tumor promoter in cancer induction. *Seminars in Cancer Biology*, 14(6):433–439, dec 2004.

[26] Yusuke Hanaki, Yuki Shikata, Masayuki Kikumori, Natsuki Hotta, Masaya Imoto, and Kazuhiro Irie. Identification of protein kinase C isozymes involved in the anti-proliferative and pro-apoptotic activities of 10-Methyl-aplog-1, a simplified analog of debromoaplysiatoxin, in several cancer cell lines. *Biochemical and Biophysical Research Communications*, 495(1):438–445, 2018.

[27] Nancy A. Sharkey and Peter M. Blumberg. Highly lipophilic phorbol esters as inhibitors of specific [^{3}H]phorbol 12,13-dibutyrate binding. *Cancer Research*, 45:19–24, 1985.

[28] Mayumi Shindo, Kazuhiro Irie, Akifumi Nakahara, Hajime Ohigashi, Hiroaki Konishi, Ushio Kikkawa, Hiroyuki Fukuda, and Paul A. Wender. Toward the identification of selective modulators of protein kinase C (PKC) isozymes: Establishment of a binding assay for PKC isozymes using synthetic C1 peptide receptors and identification of the critical residues involved in the phorbol ester binding. *Bioorganic and Medicinal Chemistry*, 9(8):2073–2081, 2001.

[29] Yoshiki Ashida, Ryo C. Yanagita, Chise Takahashi, Yasuhiro Kawanami, and Kazuhiro Irie. Binding mode prediction of aplysiatoxin, a potent agonist of protein kinase C, through molecular simulation and structure–activity study on simplified analogs of the receptor-recognition domain. *Bioorganic and Medicinal Chemistry*, 24(18):4218–4227, 2016.

[30] Takao Yamori, Akio Matsunaga, Shigeo Sato, Kanami Yamazaki, Akiko Komi, Kazuhiro Ishizu, Izumi Mita, Hajime Edatsugi, Yasuhiro Matsuba, Kimiko Takezawa, Osamu Nakanishi, Hiroshi Kohno, Yuki Nakajima, Hironori Komatsu, Toshio Andoh, and Takashi Tsuruo. Potent antitumor activity of MS-247, a novel DNA minor groove binder, evaluated by an *in vitro* and *in vivo* human cancer cell line panel. *Cancer Research*, 59(16):4042–4049, 1999.

[31] Yusuke Hanaki, Masayuki Kikumori, Sayo Ueno, Harukuni Tokuda, Nobutaka Suzuki, and Kazuhiro Irie. Structure-activity studies at position 27 of aplog-1, a simplified analog of debromoaplysiatoxin with anti-proliferative activity. *Tetrahedron*, 69(36):7636–7645, 2013.

[32] Kazuhiro Irie, Kentaro Oie, Akifumi Nakahara, Yoshiaki Yanai, Hajime Ohigashi, Paul A. Wender, Hiroyuki Fukuda, Hiroaki Konishi, and Ushio Kikkawa. Molecular basis for protein kinase C isozyme-selective binding: The synthesis, folding, and phorbol ester binding of the cysteine-rich domains of all protein kinase C isozymes. *Journal of the American Chemical Society*, 120(36):9159–9167, 1998.

[33] Yusuke Hanaki, Masayuki Kikumori, Harukuni Tokuda, Mutsumi Okamura, Shingo Dan, Naoko Adachi, Naoaki Saito, Ryo C. Yanagita, and Kazuhiro Irie. Loss of the Phenolic Hydroxyl Group and Aromaticity from the Side Chain of Anti-Proliferative 10-Methyl-aplog-1, a Simplified Analog of Aplysiatoxin, Enhances Its Tumor-Promoting and Proinflammatory Activities. *Molecules*, 22(4):631, apr 2017.

[34] Anne Monks, Dominic Scudiero, Philip Skehan, Robert Shoemaker, Kenneth Paull, David Vistica, Curtis Hose, John Langley, Paul Cronise, Anne Vaigro-Wolff, Marcia Gray-Goodrich, Hugh Campbell, Joseph Mayo, and Michael Boyd. Feasibility of a High-Flux Anticancer Drug Screen Using a Diverse Panel of Cultured Human Tumor Cell Lines. *JNCI: Journal of the National Cancer Institute*, 83(11):757–766, jun 1991.