Synthesis, Conformation, and Biological Activities of a Des-A-Ring Analog of 18-Deoxy-Aplog-1, a Simplified Analog of Debromoaplysiatoxin

Yoshiki Ashida[a], Ryo C. Yanagita[b,*], Yasuhiro Kawanami[b], Mutsumi Okamura[c], Shingo Dan[c], Kazuhiro Irie[d]

[a]Department of Applied Bioresource Science, The United Graduate School of Agricultural Sciences, Ehime University (Kagawa University), Kagawa 761-0795, Japan
[b]Department of Applied Biological Science, Faculty of Agriculture, Kagawa University, Kagawa 761-0795, Japan
[c]Division of Molecular Pharmacology, Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, 3-8-31 Ariake, Koto, Tokyo 135-8550, Japan
[d]Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan

Corresponding Author
*(R.C.Y.) E-mail: yanagita.ryo@kagawa-u.ac.jp
ORCID
Ryo C. Yanagita: 0000-0002-9217-5507

Synthesis, Conformation, and Biological Activities of a Des-A-Ring Analog of 18-Deoxy-Aplog-1, a Simplified Analog of Debromoaplysiatoxin
Yoshiki Ashida, Ryo C. Yanagita, Yasuhiro Kawanami, Mutsumi Okamura, Shingo Dan, Kazuhiro Irie
Heterocycles, Volume 99, Issue 18, 2019, Pages 942-957
https://doi.org/10.3987/COM-18-S(F)60

Dedicated to Prof. Tohru Fukuyama on the occasion of his 70th birthday.

ABSTRACT

10-Me-Aplog-1 as a simplified analog of tumor-promoting debromoaplysiatoxin and a potent activator of protein kinase C (PKC) is a promising chemotherapeutic agent. In this study, we synthesized a des-A-ring analog (**4**) of 18-deoxy-aplog-1 as a synthetically-accessible analog. Compound 4 retained the conformation of the PKC-recognition part of aplogs. Moderate affinity for conventional PKC isozymes (α, β, γ) and anti-proliferative activity against NCI-H460 (lung) and MKN45 (stomach) human cancer cell lines were observed. The results suggest that 4 could serve as a lead compound for the development of conventional PKC isozyme-selective chemotherapeutic agents.

1 INTRODUCTION

Aplysiatoxin (ATX) and debromoaplysiatoxin (DAT) that are naturally-occurring tumor promoters isolated from sea hare *Stylocheilus* longicauda[1] and cyanobacteria,[2] are potent activators of protein kinase C (PKC).[3] PKC is a family of serine/threonine kinase that plays important roles in intracellular signal transduction, such as proliferation, differentiation, and apoptosis.[4] Upon ATX and DAT binding to tandem C1 domains in the regulatory region of the eight isozymes of conventional PKC (α, βI, βII, γ) and novel PKC (δ, ε, η, θ), these isozymes translocate to the lipid membrane fraction, leading to their conformational change and activation.[5] Although ATX and DAT show great anti-proliferative activity against several human cancer cell lines,[6] their therapeutic applications are limited owing to their potent tumor-promoting and pro-inflammatory activities *in vivo*.[7] 10-Me-Aplog-1 (Figure 1), synthesized as a simplified analog of DAT in our previous study, shows anti-proliferative activity comparable to DAT, which has little tumor-promoting and pro-inflammatory activities.[8] Thus, 10-Me-aplog-1 is expected as a potential chemotherapeutic compound.

Even so, a synthetically-accessible analog is still needed because the synthesis of 10-Me-aplog-1 requires a longest linear sequence of 23 steps.[9] To achieve further simplification and shortening of the synthetic steps, we focused on spiroketal moiety in 10-Me-aplog-1. The macrocyclic structure of 10-Me-aplog-1 can be divided into three regions based on their functions (Figure 1),[10,11] *i.e.*, a recognition domain (RD) at position 1 and positions 25–28, which plays an essential role in the recognition of PKC C1 domains; a conformation controlling unit (CCU) at positions 2–11; and a hydrophobic side chain at position 11. Among these regions, the spiroketal moiety in CCU is considered to play a pivotal role in controlling the conformation of RD and provide bulkiness to cover a binding cleft in the receptor.

To date, there have been mainly two studies focusing on the modification of the spiroketal structure in DAT.[12,13] Kishi *et al*. synthesized a C-substituted oxaspiro undecane analog of DAT (**1**, Figure 1).[12] Our group recently synthesized 4-*O*-substituted simplified analogs of DAT (**2** and 3-*epi*-**2**, Figure 1).[13] However, the simplification of the spiro structure has not been reported, except for Hoffmann *et al*.; they attempted to reproduce the conformation of the macrocycle ring of ATX using a cyclohexane moiety (**3**, Figure 1).[11] Here, we report the synthesis, conformational analysis, and biological activities of the des-A-ring analog (**4**, Figure 1) of 18-deoxy-aplog-1.[14] Synthesis of **4** was achieved in a longest linear sequence of 11 steps, which is less than half of that of 10-Me-Aplog-1 (Figure 1). The conformation of **4** is slightly different from 18-deoxy-aplog-1 with the spiroketal structure. Its affinity for C1B domains of novel PKCs was several times weaker than that of 18-deoxy-aplog-1. However, **4** retained affinity for C1A domains of conventional PKCs and anti-proliferative activity for some human cancer cell lines.

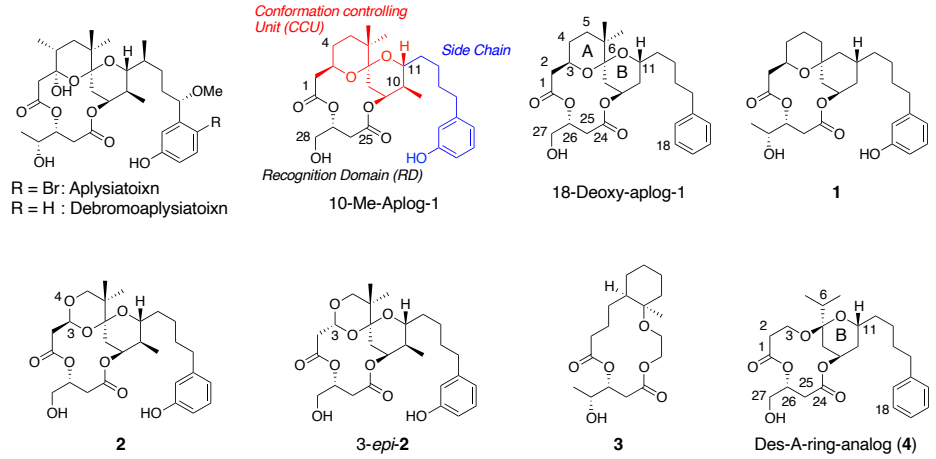


Figure 1: Structures of aplysiatoxins and their simplified analogs.

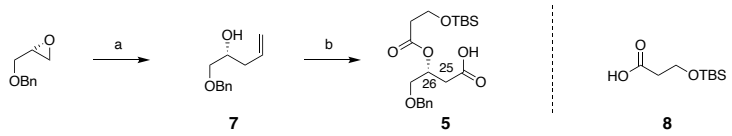
2 RESULTS AND DISCUSSION

2.1 Synthesis of des-A-ring analog (4)

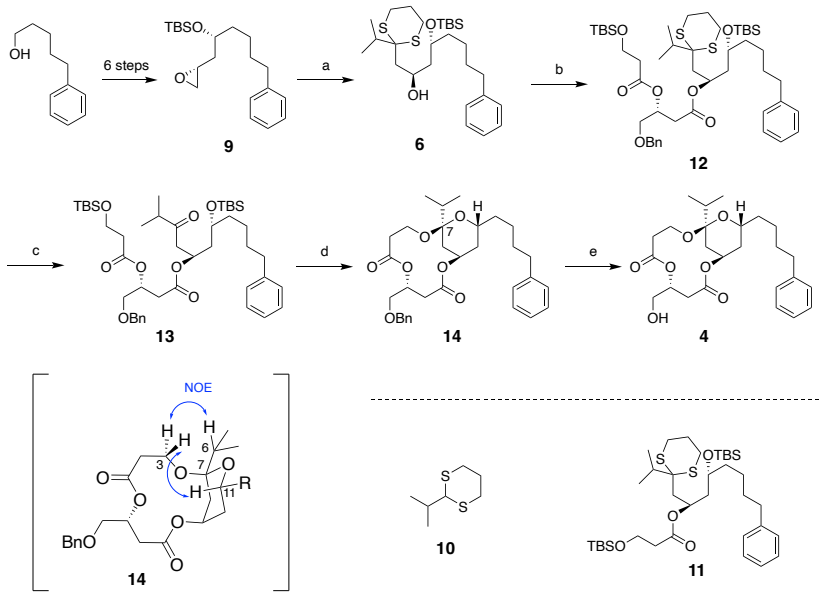
In a retrosynthetic analysis, we envisioned that **4** could be constructed via esterification of a carboxylic acid **5** and an alcohol **6** followed by macroketalization. The synthesis of the carboxylic acid unit **5** began with the coupling of (*R*)-benzylglycidyl ether with vinylmagnesium bromide to give a known alcohol **7**,[15] which was followed by esterification with a known carboxylic acid **8**[16] and oxidative cleavage of a double bond (scheme 1). The alcohol unit **6** was generated through the coupling of 2-isopropyl-1,3-dithiane[17] (**10**) and a known epoxide **9** (scheme 2), which was derived from 5-phenylpentan-1-ol (39% in six steps) as reported previously.[18] Esterification of **5** with **6** was performed via Yamaguchi's method using triethylamine as a base.[19] However, this reaction only provided an undesired byproduct **11**. We assumed that **11** was generated via β-elimination at positions 25–26 of the activated carboxylic acid followed by the formation of a mixed carboxylic anhydride and acylation of **6**. Then, we applied the modified Yamaguchi's method (one-pot method)[20] using pyridine as a base to yield the desired ester **12** in 85%. Next, a ketone **13** was synthesized via the deprotection of the dithiane group under oxidative condition using silver nitrate and *N*-chlorosuccinimide (NCS).[21] A ketal **14** was provided in a single diastereomer via simultaneous deprotection of two silyl groups and macrocyclization using HF·pyridine.[22] The relative configuration at position 7 in **14** was determined by the nuclear Overhauser effect (NOE) experiment in $CDCl_3$ (scheme 2 and Supporting Information). Finally, the deprotection of the benzyl group via catalytic hydrogenation with an addition of basic alumina[23] produced **4** from 5-phenylpentan-1-ol in a longest linear sequence of 11 steps, with an overall yield of 7.4% (scheme 2).

2.2 Conformational analysis of 4

First, we performed the conformational analysis of 4 through NMR experiments and compared its conformation with that of 18-deoxy-aplog-1[14] predicted from crystal and solution structures of aplysiatoxins.[24,25] Figure 2A (right panel) shows three-dimensional (3D) structures of a spiroketal moiety in 18-deoxy-aplog-



Scheme 1. Synthesis of carboxylic acid unit 5.
(a) 1 M vinylmagnesium bromide in THF, CuI, THF, −20 °C; (b) (i) **8**, 2,4,6-trichlorobenzoyl chloride, Et_3N, DMAP, CH_2Cl_2; (ii) $KMnO_4$, $NaIO_4$, *t*-BuOH, pH 7.2 buffer, 66% in three steps.



Scheme 2. Synthesis of des-A-ring analog **4**.
(a) **10**, *n*-BuLi, THF, 0 °C, 90%; (b) **5**, 2,4,6-trichlorobenzoyl chloride, pyridine, DMAP (cat.), THF, 85%, (c) NCS, 2,6-lutidine, $AgNO_3$, CH_3CN, H_2O, acetone, 0 °C, 86%; (d) HF·pyridine, THF, −60 °C to 3 °C; (e) H_2, Pd/C, basic alumina, THF, 29% in two steps.

1. Dihedral angles along C1–C2–C3–O–C7–O are in *gauche*$^-$, *anti*, and *anti* conformations. The NOESY experiment in $CDCl_3$ revealed correlations between H3α and H6, as well as H3β and H11 in **4** (Figure 2A, left. The NOESY spectra are in Supporting Information). Conversely, H3 in 18-deoxy-aplog-1 was spatially separated from H11 (Figure 2A, right), indicating that the conformation of this part in **4** is different from that of 18-deoxy-aplog-1. The NOE correlation between H3 and H11 was also observed in 3-*epi*-**2**,[13] which implies that **4** adopted a conformation corresponding to that of 3-*epi*-**2**. Dihedral angles along C1–C2–C3–O–C7–O are in *gauche*$^+$, *anti*, and *gauche*$^+$ conformations. The coupling constants of H25–H26 in RD of **4** ($J_{25,26}$ = 11.9 and 2.1 Hz) are similar to those of 18-deoxy-aplog-1 ($J_{25,26}$ = 11.5 and 3.4 Hz).[14] Thus, the dihedral angle at positions 25–26 in **4** is similar to that in 18-deoxy-aplog-1.

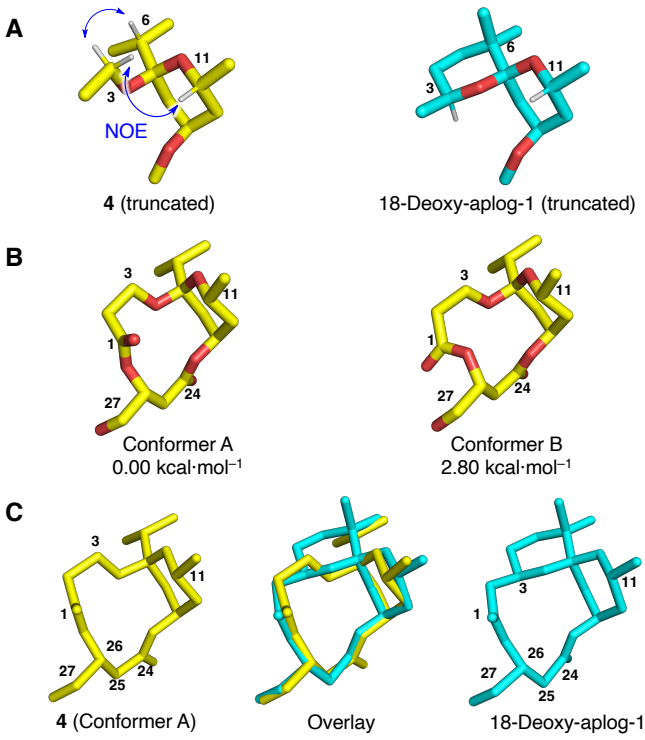


Figure 2: Three-dimensional (3D) structures of **4** and 18-deoxy-aplog-1. (A) Left: a partial structure of **4** at positions 2–3 and 6–12 part. Blue double arrowed curves represent NOE correlations. Right: a partial structure of 18-deoxy-aplog-1 at positions 2–12 part. Stick model colored yellow or cyan (carbon) and red (oxygen). (B) Possible conformers of a macrolactone core of 4 and their relative energies at the ωB97X-D/6-31G* level. Stick model colored yellow (carbon) and red (oxygen). (C) Left: a 3D structure of the macrolactone core in **4** (yellow). Right: a structure of the macrolactone core in 18-deoxy-aplog-1[14] (cyan) predicted from a crystal and solution structure of aplysiatoxins.[24,25] Center: overlay of both structures.

Next, we conducted a computational conformational search followed by the density functional theory (DFT) calculation. An aromatic side chain at position 11 was replaced with a methyl group to simplify the calculation. A set of possible conformation of the macrolactone core structure of **4** was generated using

the simulated annealing method. Among them, two conformers A and B were consistent with the NMR data. The only difference between conformers A and B is the orientation of the carbonyl groups at position 1 (Figure 2B). The two structures were pre-optimized using the molecular mechanics method with the MMFF94s force field.[26] The final DFT geometry optimizations were performed at the ωB97X-D/6-31G* level.[27] The difference in the energy between conformers A and B is 2.80 kcal·mol^{-1} (Figure 2B), which suggests that **4** existed dominantly in conformer A in $CDCl_3$. An overlay between conformer A of **4** and the putative conformation of 18-deoxy-aplog-1 shows good agreement between their conformations of RD. On the other hand, dihedral angles along C1–C2–C3–O–C7–O and the spatial orientation of a dimethyl group at position 6 are different from those of 18-deoxy-aplog-1 (Figure 2C).

2.3 Binding ability of 4 for C1 domains of PKC isozymes

Following the conformational analysis, we evaluated the ability of **4** to bind to C1 domains of PKC isozymes. Synthetic C1A and C1B peptides[28] of conventional and novel PKC isozymes were used, respectively, because tumor promoters, including ATX, mainly bind to these domains.[29–32] The concentration required to cause 50% inhibition (IC_{50}) of [^{3}H]phorbol 12,13-dibutyrate (PDBu) was measured using a competitive binding assay.[33] Affinity for each C1 peptide is expressed as the K_i value calculated from the IC_{50} value of each ligand and the dissociation constant (K_d) of [^{3}H]PDBu, as reported by Goldstein and Barrett.[34]

Table 1 lists the K_i values of **4**, 18-deoxy-aplog-1, **2**, and 3-*epi*-**2** for conventional and novel PKC isozymes. A recent study found that PKCα and δ isozymes were predominantly expressed in many cancer cell lines and were involved in the anti-proliferative activity of 10-Me-aplog-1.[35] Binding abilities of **4** for C1A domains of conventional PKCs were almost equal to those of 18-deoxy-aplog-1. By contrast, the binding abilities of **4** for C1B domains of novel PKCs were around 5–10 times weaker than those of 18-deoxy-aplog-1. Given that 3-*epi*-**2**, which would adopt conformation similar to that of **4**, showed decreased binding ability for δ-C1B compared to **2** which has same conformation as 18-deoxy-aplog-1,[13] the conformation of positions 2–7 in aplogs might be important in the recognition of C1B domains of novel PKCs.

The hydrophobicity of PKC ligands is also an important factor for their binding abilities because the insertion to the phospholipid bilayer membrane is required for PKC ligands to form a stable complex with the protein. To estimate the hydrophobicity, a log *P* value of **4** was evaluated via the HPLC method as recommended by OECD.[36,37] The hydrophobicity of **4** (log *P*, 4.3) is lower than that of 18-deoxy-aplog-1 (log *P*, 4.8).[38] This result implies that not only the ring conformation but also hydrophobicity at position 4 and/or 5 might be more important for the recognition of novel PKCs than that for conventional PKCs.

Table 1: Values of K_i for the inhibition of [^{3}H]PDBu binding of by **4**, 18-deoxy-aplog-1, **2**, and 3*epi*-**2**

	K_i (nM)						
	Conventional PKC			Novel PKC			
compound	α-C1A	β-C1A	γ-C1A	δ-C1B	ε-C1B	η-C1B	θ-C1B
4	100 (0)[c]	160 (10)	50 (10)	130 (20)	240 (0)	60 (0)	70 (0)
18-deoxy-aplog-1[a]	120	140	80	9.8	37	12	8.1
2[b]	22	NT[d]	NT	6.8	NT	NT	NT
3-*epi*-**2**[b]	22	NT	NT	13	NT	NT	NT

[a] Cited from Ref. [14], [b] Cited from Ref. [13], [c] Standard deviation of at least two independent experiments, [d] Not tested.

2.4 Anti-proliferative activity of 4

Finally, we evaluated the anti-proliferative activity of **4** against a panel of 39 human cancer cell lines (JFCR39) reported by Yamori *et al.*[39] Growth inhibitory activity was expressed as GI_{50} (M), *i.e.*, the concentration required to inhibit cell growth by 50% compared with untreated control.

Table 2 lists log GI_{50} values of **4** and 18-deoxy-aplog-1 for 11 cancer cell lines whose log GI_{50} values of 18-deoxy-aplog-1 were less than -5.00 (the values for the other cancer cell lines are provided in Supporting Information). Compound **4** did not show significant anti-proliferative activity against most of cell lines (log $GI_{50} > -5.0$), which can be attributed to the more-than-ten-fold reduction in the ability to bind to PKCδ. However, **4** exhibited significant anti-proliferative activity against NCI-H460 and MKN45 cells. In particular, the activity of **4** against NCI-H460 (log GI_{50}, -5.53) was comparable to that of 18-deoxy-aplog-1 (log GI_{50}, -5.83). This result suggests that conventional PKCs, rather than novel PKCs, were involved in the anti-proliferative activity against NCI-H460 and MKN45. The growth inhibition assay also revealed that efficacy profile of **4** for a panel of 39 cancer cell lines was similar to that of 18-deoxy-aplog-1 (Pearson correlation coefficient $r = 0.719$), suggesting that the mode of action of **4** did not greatly change from that of 18-deoxy-aplog-1.

Table 2: Growth inhibitory activities of **4** and 18-deoxy-aplog-1 against several human cancer cell lines

		log GI_{50} (M)	
Cancer type	Cell line	**4**	18-Deoxy-aplog-1[a]
Breast	HBC-4	−4.98	−6.28
	MDA-MB-231	−4.90	−5.67
	BSY-1	−4.78	−5.17
CNS	SF-295	−4.94	−5.14
Colon	HCC2998	−4.86	−5.53
Lung	NCI-H460	−5.53	−5.83
	A549	−4.92	−5.49
Melanoma	LOX-IMVI	−4.99	−5.17
Stomach	St-4	−4.87	−6.05
	MKN45	−5.21	−6.09
Prostate	PC-3	−4.85	−5.26

[a] Cited from Ref. [14].

3 CONCLUSION

In conclusion, we successfully reduced the number of synthetic steps of 18-deoxy-aplog-1, without losing affinity for C1A domains of conventional PKCs by the removal of the A-ring. Conformational analysis of the des-A-ring analog **4** revealed that the conformation of RD of aplogs was hardly affected by the removal of the A-ring. This structural change selectively decreased the affinity for novel PKCs, which is somewhat surprising, because nearly all structural modifications of aplogs in our previous studies increased the isozyme selectivity toward novel PKCs. Although the reason why **4** retained affinity for C1A domains of conventional PKCs is still not clear, the spatial orientation of a dimethyl group at position 6 and/or hydrophobicity at positions 4–5 might be responsible for this phenomenon. Because **4** showed selective anti-proliferative activity against NCI-H460 (lung) and MKN45 (stomach) human cancer cell lines, it could serve as a lead compound for the development of selective chemotherapeutic agents for such types

of cancer. Furthermore, the installation of a methyl group at positions 10 and/or 12 would be promising to enhance biological activities of **4** as exemplified by 10-Me-Aplog-1 and 10,12-diMe-Aplog-1.[8,40] The structural optimization of **4** is currently under progress.

4 EXPERIMENTAL

4.1 General remarks

The following spectroscopic and analytical instruments were used: Digital Polarimeter, JASCO P-1010 (JASCO, Tokyo, Japan); NMR (^{1}H-1D, ^{13}C-1D, COSY, and NOESY), JEOL JNM-ECA 600 (Jeol, Japan, reference TMS); HPLC, JASCO PU-4086 semi-preparation pump with a JASCO PV-4075 UV/Vis Detector (JASCO, Tokyo, Japan); HR-ESI-TOF-MS, Xevo G2-XS (Waters, Tokyo, Japan). HPLC was carried out on a YMC-pack ODS-AM AM12S05-1520WT (YMC, Kyoto, Japan), YMC-pack ODS-A AA12S05-1510WT (YMC, Kyoto, Japan), or YMC-pack ODS-A AA12S05-1006WT (YMC, Kyoto, Japan). Wakogel® C-200 and C-300 (silica gel, FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) were used for column chromatography. [^{3}H]PDBu (17.16 Ci/mmol) was purchased from PerkinElmer Life Science Research Products (Boston, MA, US). The PKC C1 peptides were synthesized as reported previously.[28] All other chemicals and reagents were purchased from chemical companies and used without purification.

4.2 Synthetic procedures

4.2.1 (*R*)-4-(Benzyloxy)-3-((3-((*tert*-butyldimethylsilyl)oxy)propanoyl)oxy)butanoic acid (5).

To a suspension of (*R*)-benzylglycidyl ether (168.2 mg, 1.02 mmol) and CuI (194.3 mg, 1.02 mmol) in THF (6.8 mL) was added 1 M vinylmagnesium bromide in THF (4.1 mL, 4.08 mmol) quickly at −20 °C under an Ar atmosphere. After stirring of 25 min, the dark-green–black mixture was quenched with sat. NH_4Cl aq. (48 mL). The resulting mixture was diluted with EtOAc (40 mL) and was stirred at rt for 1.5 h. The organic layer was separated and the aqueous layer was extracted with EtOAc (40 mL × 2). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to afford a secondary alcohol **7**[15] (216.7 mg) as a pale yellow oil, which was taken to next step without further purification.

To a solution of a carboxylic acid **8**[16] (93.7 mg, 0.458 mmol) and Et_3N (72 µL, 0.518 mmol) in CH_2Cl_2 (2.1 mL) was added 2,4,6-trichlorobenzoyl chloride (81 µL, 0.518 mmol) at rt under an Ar atmosphere. After stirring of 4 h, the resulting mixture was added to a solution of **7** (58.6 mg) and DMAP (74.5 mg, 0.610 mmol) in CH_2Cl_2 (2.8 mL). The mixture was stirred for 1.5 h, and the reaction was quenched with H_2O (12 mL). The resulting mixture was extracted with EtOAc (20 mL + 12 mL × 2). The combined organic layers were washed with sat. $NaHCO_3$ aq. and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, 5% EtOAc in hexane) to afford an ester (106.3 mg) as a pale yellow oil, which was taken to next step without further purification.

To a suspension of $NaIO_4$ (481.2 mg, 2.25 mmol) in 50 mM phosphate buffer (pH 7.2, 23.1 mL) was added $KMnO_4$ (44.4 mg, 0.281 mmol) in one portion. After stirring of 10 min at rt under an Ar atmosphere, the mixture was added to a solution of the ester (106.3 mg) in *t*-BuOH (23.1 mL). The reaction mixture was stirred at rt for 1 h, and the reaction was quenched with $Na_2S_2O_3$ (151 mg). The resulting mixture was poured into H_2O (40 mL), and the aqueous layer was extracted with EtOAc (150 mL + 50 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, 20% EtOAc in hexane containing 0.1% AcOH) to afford a carboxylic acid **5** (72.5 mg, 0.183 mmol, 66% in three steps) as a colorless oil. $[\alpha]_D$: +11° (*c* 0.99, $CHCl_3$, 26.4 °C). ^{1}H NMR (600 MHz, 297 K, $CDCl_3$, 0.0484 M): δ 0.05 (6H, m), 0.87 (9H,

m), 2.53 (2H, t, *J* = 6.5 Hz), 2.73 (1H, dd, *J* = 16.7, 7.2 Hz), 2.79 (1H, dd, *J* = 16.5, 5.8 Hz), 3.57 (1H, dd, *J* = 10.5, 4.9 Hz), 3.65 (1H, dd, *J* = 10.5, 4.7 Hz), 3.88 (2H, m), 4.51 (1H, d, *J* = 12.0 Hz), 4.56 (1H, d, *J* = 12.0 Hz), 5.38 (1H, m), 7.27–7.36 (5H, m) ppm. ^{13}C NMR (150 MHz, 298 K, $CDCl_3$, 0.0484 M): δ −5.4 (2C), 18.2, 25.8 (3C), 35.6, 38.0, 58.9, 68.8, 70.0, 73.3, 127.7 (2C), 127.8, 128.4 (2C), 137.7, 171.0, 175.2 ppm. HR-ESI-MS: *m*/*z* = 419.1872 ($[MNa]^+$, calcd for $C_{20}H_{32}O_6SiNa$, 419.1866).

4.2.2 (2*R*,4*R*)-4-((*tert*-Butyldimethylsilyl)oxy)-1-(2-isopropyl-1,3-dithian-2-yl)-8-phenyloctan-2-ol (6).

To a solution of 2-isopropyl-1,3-dithiane[17] (**10**, 230.5 mg, 1.42 mmol) in THF (7.7 mL) was added 1.6 M *n*-BuLi in hexane (890 μL, 1.42 mmol) at 0 °C under an Ar atmosphere. After stirring of 1 h, to the resulting mixture was added a solution of an epoxide **9**[18] (118.3 mg, 0.354 mmol) in THF (2.8 mL). After further stirring of 1.5 h, the reaction was quenched with sat. NH_4Cl aq. (15 mL). The resulting mixture was diluted with H_2O (5 mL), and 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% EtOAc in hexane) to afford an alcohol **6** (159.0 mg, 0.320 mmol, 90%) as a colorless oil. $[\alpha]_D$: +1.4° (*c* 0.29, $CHCl_3$, 26.5 °C). ^{1}H NMR (600 MHz, 297 K, $CDCl_3$, 0.0109 M): δ 0.05 (3H, s), 0.08 (3H, s), 0.88 (9H, s), 1.06 (3H, d, *J* = 6.7 Hz), 1.20 (3H, d, *J* = 6.7 Hz), 1.33–1.68 (7H, m), 1.72 (1H, m), 1.90 (1H, m), 1.96–2.03 (2H, m), 2.21 (1H, dd, *J* = 15.5, 8.6 Hz), 2.36 (1H, sep, *J* = 6.7 Hz), 2.61 (2H, t, *J* = 7.8 Hz), 2.76–2.89 (3H, m), 2.96 (1H, m), 3.70 (1H, s), 3.91 (1H, m), 4.11 (1H, m), 7.16 (3H, m), 7.26 (2H, m) ppm. ^{13}C NMR (150 MHz, 298 K, $CDCl_3$, 0.0109 M): δ −4.3 (2C), 17.5, 18.1, 18.3, 24.9, 25.0, 25.6, 25.9 (3C), 26.1, 31.7, 34.4, 36.0, 36.6, 42.5, 45.2, 57.5, 66.1, 70.4, 125.6, 128.2 (2C), 128.4 (2C), 142.7 ppm. HR-ESI-MS: *m*/*z* = 519.2769 ($[MNa]^+$, calcd for $C_{27}H_{48}O_2S_2SiNa$, 519.2763).

4.2.3 (*R*)-(2*R*,4*R*)-4-((*tert*-Butyldimethylsilyl)oxy)-1-(2-isopropyl-1,3-dithian-2-yl)-8-phenyloctan-2-yl 4-(benzyloxy)-3-((3-((*tert*-butyldimethylsilyl)oxy)propanoyl)oxy)butanoate (12).

To a solution of **6** (60.0 mg, 0.121 mmol), **5** (72.2 mg, 0.182 mmol), pyridine (54 μL, 0.666 mmol), and DMAP (1.5 mg, 12.1 μmol) in THF (650 μL) was added quickly 2,4,6-trichlorobenzoyl chloride (57 μL, 0.363 mmol) in one portion at rt under an Ar atmosphere. After stirring of 28 h, the reaction was quenched with sat. $NaHCO_3$ aq. (2.7 mL) at 0 °C. The resulting mixture was extracted with EtOAc (4 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, 5% EtOAc in hexane) to afford an ester **12** (89.8 mg, 0.130 mmol, 85%) as a colorless oil. $[\alpha]_D$: +16° (*c* 0.31, $CHCl_3$, 26.5 °C). ^{1}H NMR (600 MHz, 297 K, $CDCl_3$, 0.0192 M): δ 0.02 (3H, s), 0.04 (6H, s), 0.06 (3H, s), 0.87 (9H, s), 0.88 (9H, s), 1.09 (3H, d, *J* = 6.7 Hz), 1.12 (3H, d, *J* = 6.7 Hz), 1.31–1.51 (3H, m), 1.56–1.68 (3H, m), 172–1.86 (3H, m), 1.92 (1H, m), 2.19 (1H, sep, *J* = 6.7 Hz), 2.29 (1H, dd, *J* = 15.9, 2.9 Hz), 2.34 (1H, t, *J* = 15.9, 7.3 Hz), 2.51 (2H, m), 2.61 (3H, m), 2.69 (3H, m), 2.76–2.88 (2H, m), 3.59 (1H, dd, *J* = 10.5, 4.3 Hz), 3.64 (1H, dd, *J* = 10.8, 4.8 Hz), 3.67 (1H, m), 3.83–3.91 (2H, m), 4.50 (1H, d, *J* = 12.0 Hz), 4.54 (1H, d, *J* = 12.0 Hz), 5.31 (1H, m), 5.39 (1H, m), 7.16 (3H, m), 7.24–7.35 (7H, m) ppm. ^{13}C NMR (150 MHz, 298 K, $CDCl_3$, 0.0192 M): δ −5.4 (2C), −4.5, −4.3, 17.9, 17.9, 18.1, 18.2, 25.0, 25.1, 25.7, 25.9 (3C), 25.9 (3C), 31.6, 34.2, 36.0, 36.0, 36.1, 38.0, 40.1, 43.5, 57.5, 59.0, 69.2, 69.4 (2C), 70.3, 73.3 (2C), 125.6, 127.6 (2C), 127.7, 128.2 (2C), 128.4 (2C), 128.4 (2C), 138.0, 142.7, 169.4, 170.8 ppm. HR-ESI-MS: *m*/*z* = 897.4655 ($[MNa]^+$, calcd for $C_{47}H_{78}O_7S_2Si_2Na$, 897.4625).

4.2.4 (*R*)-(5*R*,7*R*)-7-((*tert*-Butyldimethylsilyl)oxy)-2-methyl-3-oxo-11-phenylundecan-5-yl 4-(benzyloxy)-3-((3-((*tert*-butyldimethylsilyl)oxy)propanoyl)oxy)butanoate (13).

To a stirred solution of *N*-chlorosuccinimide (142.9 mg, 1.07 mmol), $AgNO_3$ (278.6 mg, 1.64 mmol), and 2,6-lutidine (251 μL, 2.17 mmol) in CH_3CN (27.4 mL) and H_2O (11.4 mL) was added a solution of **12** (66.8 mg, 0.0763 mmol) in acetone (9.3 mL) at 0 °C. After stirring of 20 min, the reaction mixture was diluted with EtOAc (70 mL), and the reaction was quenched with sat. $NaHCO_3$ aq. and sat. $Na_2S_2O_3$ aq. (1:1, 70 mL). The organic layer was separated and the aqueous layer was extracted with EtOAc (90 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, 7% EtOAc in hexane) to afford a ketone **13** (51.4 mg, 0.0655 mmol, 86%) as a colorless oil. $[\alpha]_D$: +6.3° (*c* 0.26, $CHCl_3$, 26.6 °C). ^{1}H NMR (600 MHz, 296 K, $CDCl_3$, 0.0229 M): δ 0.01 (3H, s), 0.03 (3H, s), 0.04 (6H, s), 0.86 (9H, s), 0.87 (9H, s), 1.06 (6H, d, *J* = 7.1 Hz), 1.28–1.48 (3H, m), 1.52–1.63 (3H, m), 1.68 (1H, m), 1.77 (1H, m), 2.52 (3H, m), 2.60 (3H, m), 2.67 (1H, dd, *J* = 15.9, 6.0 Hz) 2.71 (1H, br.s), 2.73 (1H, br.d, *J* = 1.3 Hz), 3.55 (1H, dd, *J* = 10.4, 4.6 Hz), 3.61 (1H, dd, *J* = 10.4, 4.8 Hz), 3.68 (1H, m), 3.83–3.91 (2H, m), 4.50 (1H, d, *J* = 12.0 Hz), 4.55 (1H, d, *J* = 12.0 Hz), 5.32 (1H, m), 5.35 (1H, m), 7.16 (3H, m), 7.26 (3H, m), 7.33 (4H, m) ppm. ^{13}C NMR (150 MHz, 297 K, $CDCl_3$, 0.0229 M): δ −5.4, −4.5, −4.5 (2C), 17.9, 18.0, 18.0, 18.2, 24.9, 25.8 (3C), 25.9 (3C), 31.6, 35.9, 36.2, 36.4, 38.0, 41.1, 41.3, 44.7, 58.9, 68.5, 69.1, 69.2, 70.2, 73.3, 125.6, 127.6 (2C), 127.7, 128.2 (2C), 128.4 (2C), 128.4 (2C), 137.9, 142.6, 169.4, 170.9, 211.1 ppm. HR-ESI-MS: *m*/*z* = 807.4698 ($[MNa]^+$, calcd for $C_{44}H_{72}O_8Si_2Na$, 807.4663).

4.2.5 (1*R*,5*R*,11*R*,13*R*)-5-(Hydroxymethyl)-11-isopropyl-13-(4-phenylbutyl)-2,6,10,12-tetraoxabicyclo[9.3.1]pentadecane-3,7-dione (4).

To a solution of **13** (36.6 mg, 46.6 μmol) in THF (13 mL) was added HF·pyridine (1.5 mL) at −60 °C under an Ar atmosphere and then the reaction mixture was warmed to 3 °C (kept under 5 °C). After stirring of 18 h, the reaction was quenched with sat. $NaHCO_3$ aq. (70 mL) at 0 °C. The resulting mixture was extracted with EtOAc (90 mL + 70 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, 10% EtOAc in hexane) to afford a mixture of ketal 14 and by-product, which is difficult to separate by silica gel column chromatography (13.0 mg), as a colorless oil, which was taken to next step without further purification.

Separately, to measure spectral data, the mixture of **14** and the by-product was synthesized again, and purified by reversed-phase HPLC. Compound **13** (25.0 mg, 31.8 μmol) was treated in a manner similar to that described above to afford the mixture (10.6 mg). The mixture was filtered and purified by HPLC (column: YMC-Pack ODS-AM AM12S05-1520WT, solvent: 90% MeOH/H_2O, flow rate: 8 mL/min, UV detector: 254 nm, retention time: 19.6 min) to afford **14** (6.8 mg, 12.6 μmol, 40%) as a colorless oil. $[\alpha]_D$: +93° (*c* 0.68, $CHCl_3$, 26.4 °C). ^{1}H NMR (600 MHz, 297 K, $CDCl_3$, 0.0249 M): δ 0.82 (3H, d, *J* = 6.9 Hz), 0.89 (3H, d, *J* = 6.9 Hz), 1.37 (1H, m), 1.40–1.73 (8H, m), 1.98 (1H, sep, *J* = 6.9 Hz), 2.04 (1H, dt, *J* = 14.9, 2.1 Hz), 2.37 (1H, m), 2.57 (1H, m), 2.64 (2H, t, *J* = 7.9 Hz), 2.72 (1H, dd, *J* = 17.7, 2.2 Hz), 2.85 (1H, dd, *J* = 17.7, 11.9 Hz), 3.47 (2H, m), 3.57 (1H, dd, *J* = 10.0, 4.5 Hz), 3.68 (1H, m), 3.83 (1H, m), 4.50 (1H, d, *J* = 12.0 Hz), 4.58 (1H, d, *J* = 12.0 Hz), 5.02 (1H, m), 5.55 (1H, m), 7.16 (1H, m), 7.20 (2H, m), 7.25–7.36 (7H, m) ppm. ^{13}C NMR (150 MHz, 298 K, $CDCl_3$, 0.0249 M): δ 16.3, 17.8, 25.1, 28.8, 31.7, 32.5, 34.6, 35.6, 35.7, 36.0, 37.4, 55.6, 64.4, 68.3, 68.5, 70.6, 73.4, 101.2, 125.6, 127.7 (2C), 127.8, 128.2 (2C), 128.4 (4C), 137.8, 143.0, 169.4, 172.9 ppm. HR-ESI-MS: *m*/*z* = 561.2844 ($[MNa]^+$, calcd for $C_{32}H_{42}O_7Na$, 561.2828).

To a solution of the mixture of **14** (13.0 mg) in THF (2.1 mL) was added basic alumina (8.2 mg) and Pd/C (1.5 mg) at rt under an Ar atmosphere. After stirring of 18 h at rt under a H_2 atmosphere,

Pd/C (2.0 mg) was added. The reaction mixture was vigorously stirred for 2 h at rt under a H_2 atmosphere, and filtered. The filtrate was concentrated and purified by HPLC (column: YMC-Pack ODS-AM AM12S05-1510WT, solvent: 75% MeOH/H_2O, flow rate: 3 mL/min, UV detector: 254 nm, retention time: 26.1 min) to afford **4** (6.0 mg, 13.4 μmol, 29% in two steps) as a colorless oil. $[\alpha]_D$: +89° (*c* 0.42, $CHCl_3$, 26.5 °C). ^{1}H NMR (600 MHz, 296 K, $CDCl_3$, 0.0268 M): δ 0.82 (3H, d, *J* = 7.0 Hz), 0.89 (3H, d, *J* = 7.0 Hz), 1.36–1.91 (10H, m), 1.99 (1H, sep, *J* = 7.0 Hz), 2.06 (1H, dd, *J* = 15.0, 2.2 Hz), 2.40 (1H, m), 2.60 (1H, m) 2.63 (2H, t, *J* = 7.9 Hz), 2.64 (1H, dd, *J* = 17.7, 2.1 Hz), 2.85 (1H, dd, *J* = 17.7, 11.9 Hz), 3.48 (1H, m), 3.65 (1H, dd, *J* = 11.8, 5.3 Hz), 3.69 (1H, m), 3.72 (1H, dd, *J* = 11.8, 4.4 Hz), 3.83 (1H, m), 5.03 (1H, m), 5.48 (1H, m), 7.15–7.21 (3H, m), 7.26–7.29 (2H, m) ppm. ^{13}C NMR (150 MHz, 297 K, $CDCl_3$, 0.0268 M): δ 16.3, 17.8, 25.1, 28.7, 31.7, 32.5, 34.7, 35.7 (2C), 36.0, 36.7, 55.6, 64.4 (2C), 68.7, 70.7, 101.3, 125.6, 128.2 (2C), 128.4 (2C), 142.9, 169.1, 173.7 ppm. HR-ESI-MS: m/z = 471.2354 ($[MNa]^+$, calcd for $C_{25}H_{36}O_7Na$, 471.2359).

4.3 Conformational search of 4.

The three-dimensional structure of **4** was built using Avogadro (version 1.0.3).[41] An aromatic side chain at C11 was replaced with methyl group to simplify calculation. Simulated annealing was carried out using the GROMACS program (version 2016.4)[42] with a general AMBER force field 2 (GAFF2) in the AmberTools17 package.[43] All bonds were constrained using the LINCS algorithm. The time step was set to 1 fs. The annealing temperature was initially set to 1,500 K and the temperature was kept constant for 1 ps. The temperature was linearly dropped to 100 K over 1 ps and then to 0 K over 1 ps, and kept at the same temperature for 1 ps. This 5-ps cycle was repeated 1,000 times to give conformer library. We obtained two possible conformers A and B that were consistent with the NMR data. Structures of conformers A and B were optimized using the DFT method at the level of ωB97X-D/6-31G*[27] by the Gaussian09 program.[44]

4.4 Inhibition of specific binding of [^{3}H]PDBu to the PKC C1 peptides.

The binding of [^{3}H]PDBu to C1A domains of conventional PKCs and C1B domains of novel PKCs were evaluated by the procedure of Sharkey and Blumberg[33] with modifications as reported previously[29] using 50 mM Tris–maleate buffer (pH 7.4 at 4 °C), 13.8 nM (for δ-, ε-, η-, and θ-C1B), 20 nM (for γ-C1A), or 40 nM (for α- and β-C1A) peptide, 20 nM [^{3}H]PDBu (17.16 Ci/mmol, Perkin-Elmer Life Science), 50 μg/mL 1,2-dioleoyl-*sn*-glycero-3-phospho-L-serine (Funakoshi), 3 mg/mL bovine γ-globulin (Sigma), and various concentrations of the inhibitor. Binding affinity was evaluated on the basis of the concentration required to cause 50% inhibition of the specific binding of [^{3}H]PDBu, IC_{50}, which was calculated by logit analysis using Microsoft Excel. The binding inhibition constant, K_i, was calculated by the equation of Goldstein and Barrett,[34] $K_i = IC_{50}/(2[L_{50}]/[L_0] - 1 + [L_{50}]/K_d)$, where $[L_{50}]$ and $[L_0]$ are the free concentration of [^{3}H]PDBu at 50% and 0% inhibition, respectively.

4.5 Determination of log *P* of the 4 using HPLC.

The HPLC method used to determine log *P* value of **4** was from the 'OECD GUIDELINE FOR TESTING OF CHEMCALS'.[37] A solution of 75% CH_3CN/H_2O was used for the mobile phase on a reversed-phase column YMC-pack ODS-A AA12S05-1006WT. For preparing a calibration curve, a set of six compounds, phenol (1.5), anisole (2.1), 1-naphthol (2.7), cumene (3.7), *n*-butylbenzene (4.6), and triphenylamine (5.7), was used as reference compounds. Dead time was measured using unretained formamide. All compounds were monitored with a UV detector at 220 nm.

4.6 Measurement of cell growth inhibition of 4.

A panel of 39 human cancer cell lines established by Yamori and colleagues[39] according to the NCI method with modifications was employed, and cell growth inhibitory activity was measurement as reported previously.[45] 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}) concentrations were calculated as report previously.[46] Absorbance for the control well (C) and 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 (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.

5 ACKNOWLEDGEMENTS

This work was supported by JSPS KAKENHI Grant Number JP17H06405 (Frontier Research on Chemical Communications). The JFCR39 cancer cell panel assays were performed by courtesy of Molecular Profiling Committee, Grant-in-Aid for Scientific Research on Innovative Areas "Platform of Advanced Animal Model Support" from The Ministry of Education, Culture, Sports, Science and Technology, Japan (KAKENHI JP16H06276).

The DFT calculations were performed using the Research Center for Computational Science, Okazaki, Japan.

References

[1] 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.

[2] Richard E. Moore. Toxins, anticancer agents, and tumor promoters from marine prokaryotes. *Pure and Applied Chemistry*, 54(10):1919–1934, jan 1982.

[3] Yasutomi Nishizuka. The role of protein kinase C in cell surface signal transduction and tumour promotion. *Nature*, 308:693–698, 1984.

[4] Helen J. Mackay and Christopher J. Twelves. Targeting the protein kinase C family: are we there yet? *Nature reviews. Cancer*, 7(7):554–562, 2007.

[5] John P. Arcoleo and I. Bernard Weinstein. Activation of protein kinase C by tumor promoting phorbol esters, teleocidin and aplysiatoxin in the absence of added calcium. *Carcinogenesis*, 6(2):213–217, feb 1985.

[6] Oscar F. Ramos, Maria G. Masucci, and Eva Klein. Activation of Cytotoxic Activity of Human Blood Lymphocytes by Tumor-promotinq Compounds. *Cancer Research*, 44(5):1857–1862, 1984.

[7] Hirota Fujiki and Takashi Sugimura. New Classes of Tumor Promoters: Teleocidin, Aplysiatoxin, and Palytoxin. In George Klein and Sidney Weinhouse, editors, *Advances in Cancer Research*, volume 49, pages 223–264. Academic Press, London, 1987.

[8] 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.

[9] 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.

[10] Henner Knust and Reinhard W. Hoffmann. A case study in conformation design: Learning by doing. *Chemical Record*, 2(6):405–418, 2002.

[11] Henner Knust and Reinhard W. Hoffmann. Synthesis and conformational analysis of macrocyclic dilactones mimicking the pharmacophore of aplysiatoxin. *Helvetica Chimica Acta*, 86(6):1871–1893, 2003.

[12] Robert R. Rando and Yoshito Kishi. Structural Basis of Protein Kinase C Activation by Diacylglycerols and Tumor Promoters. *Biochemistry*, 31(8):2211–2218, 1992.

[13] Koutaro Hayakawa, Yusuke Hanaki, Harukuni Tokuda, Ryo C. Yanagita, Yu Nakagawa, Mutsumi Okamura, Shingo Dan, and Kazuhiro Irie. Synthesis and Biological Activities of Acetal Analogs at Position 3 of 10-Methyl-Aplog-1, a Potential Anti-Cancer Lead Derived from Debromoaplysiatoxin. *Heterocycles*, 97(1):478–492, 2018.

[14] Ryo C. Yanagita, Hiroaki Kamachi, Keisuke Tanaka, Akira Murakami, Yu Nakagawa, Harukuni Tokuda, Hiroshi Nagai, and Kazuhiro Irie. Role of the phenolic hydroxyl group in the biological activities of simplified analogue of aplysiatoxin with antiproliferative activity. *Bioorganic and Medicinal Chemistry Letters*, 20(20):6064–6066, 2010.

[15] Carlo Bonini, Lucia Chiummiento, Maddalena Pullez, Guy Solladié, and Françoise Colobert. Convergent highly stereoselective preparation of the C12-C24 fragment of macrolactin A. *Journal of Organic Chemistry*, 69(15):5015–5022, 2004.

[16] Jun Qi, Adam R. Blanden, Susan Bane, and David G. I. Kingston. Design, synthesis and biological evaluation of a simplified fluorescently labeled discodermolide as a molecular probe to study the binding of discodermolide to tubulin. *Bioorganic and Medicinal Chemistry*, 19(17):5247–5254, 2011.

[17] Jeroen S. Dickschat, Susanne Wickel, Christoph J. Bolten, Thorben Nawrath, Stefan Schulz, and Christoph Wittmann. Pyrazine biosynthesis in corynebacterium glutamicum. *European Journal of Organic Chemistry*, 2010(14):2687–2695, 2010.

[18] 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.

[19] Junji Inanaga, Kuniko Hirata, Hiroko Saeki, Tsutomu Katsuki, and Masaru Yamaguchi. A Rapid Esterification by Means of Mixed Anhydride and Its Application to Large-ring Lactonization. *Bulletin of the Chemical Society of Japan*, 52(7):1989–1993, 1979.

[20] Ilirian Dhimitruka and John SantaLucia. Investigation of the Yamaguchi esterification mechanism. Synthesis of a Lux-S enzyme inhibitor using an improved esterification method. *Organic Letters*, 8(1):47–50, 2006.

[21] E. J. Corey and Bruce W. Erickson. Oxidative hydrolysis of 1,3-dithiane derivatives to carbonyl compounds using *N*-halosuccinimide reagents. *The Journal of Organic Chemistry*, 36(23):3553–3560, nov 1971.

[22] Paul A. Wender and Vishal A. Verma. The design, synthesis, and evaluation of C7 diversified bryostatin analogs reveals a hot spot for PKC affinity. *Organic Letters*, 10(15):3331–3334, 2008.

[23] Rie Namme, Takashi Mitsugi, Hideyo Takahashi, Moto Shiro, and Shiro Ikegami. Synthesis of 1-deoxyhept-2-ulosyl-glycono-1,5-lactone utilizing α-selective *O*-glycosidation of 2,6-anhydro-1-deoxy-D-hept-1-enitols. *Tetrahedron*, 62(39):9183–9192, 2006.

[24] Richard E. Moore, Adrian J. Blackman, Chad E. Cheuk, Jon S. Mynderse, Gayle K. Matsumoto, Jon Clardy, Ronald W. Woodard, and J. Cymerman Craig. Absolute Stereochemistries of the Aplysiatoxins and Oscillatoxin A. *The Journal of Organic Chemistry*, 49(13):2484–2489, 1984.

[25] Hideshi Nakamura, Yoshito Kishi, Maria A. Pajares, and Robert R. Rando. Structural basis of protein kinase C activation by tumor promoters. *Proceedings of the National Academy of Sciences of the United States of America*, 86(24):9672–9676, 1989.

[26] Thomas A. Halgren. Merck molecular force field. V. Extension of MMFF94 using experimental data, additional computational data, and empirical rules. *Journal of Computational Chemistry*, 641(1996):616–641, 1996.

[27] Jeng-Da Chai and Martin Head-Gordon. Long-range corrected hybrid density functionals with damped atom–atom dispersion corrections. *Physical Chemistry Chemical Physics*, 10(44):6615, 2008.

[28] 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, sep 1998.

[29] 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.

[30] Andrew F. G. Quest and Robert M. Bell. The regulatory region of protein kinase Cγ. Studies of phorbol ester binding to individual and combined functional segments expressed as glutathione S-transferase fusion proteins indicate a complex mechanism of regulation by phospholipids, phorbol esters,. *The Journal of Biological Chemistry*, 269(31):20000–20012, 1994.

[31] Arathi Raghunath, Mia Ling, and Christer Larsson. The catalytic domain limits the translocation of protein kinase Cα in response to increases in Ca^{2+} and diacylglycerol. *Biochemical Journal*, 370(3):901–912, mar 2003.

[32] Zoltan Szallasi, Krisztina Bogi, Shiva Gohari, Tamas Biro, Peter Acs, and Peter M. Blumberg. Nonequivalent roles for the first and second zinc fingers of protein kinase Cδ. Effect of their mutation on phorbol ester-induced translocation in NIH 3T3 cells. *The Journal of Biological Chemistry*, 271(31):18299–18301, 1996.

[33] 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.

[34] A. Goldstein and Ronald W. Barrett. Ligand dissociation constants from competition binding assays: errors associated with ligand depletion. *Molecular Pharmacology*, 31(6):603–609, 1987.

[35] 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.

[36] W. Klein, W. Kördel, M. Weiß, and H.J. Poremski. Updating of the OECD Test Guideline 107 "partition coefficient N-octanol/water": OECD Laboratory Intercomparison Test on the HPLC method. *Chemosphere*, 17(2):361–386, jan 1988.

[37] OECD iLibrary. Test No. 117: Partition Coefficient (*n*-octanol/water), HPLC Method, 2014.

[38] Hiroaki Kamachi, Keisuke Tanaka, Ryo C. Yanagita, Akira Murakami, Kazuma Murakami, Harukuni Tokuda, Nobutaka Suzuki, Yu Nakagawa, and Kazuhiro Irie. Structure-activity studies on the side chain of a simplified analog of aplysiatoxin (aplog-1) with anti-proliferative activity. *Bioorganic and Medicinal Chemistry*, 21(10):2695–2702, 2013.

[39] 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.

[40] 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.

[41] Marcus D. Hanwell, Donald E. Curtis, David C. Lonie, Tim Vandermeersch, Eva Zurek, and Geoffrey R. Hutchison. Avogadro: an advanced semantic chemical editor, visualization, and analysis platform. *Journal of Cheminformatics*, 4:17, 2012.

[42] David Van Der Spoel, Erik Lindahl, Berk Hess, Gerrit Groenhof, Alan E. Mark, and Herman J. C. Berendsen. GROMACS: Fast, flexible, and free. *Journal of Computational Chemistry*, 26(16):1701–1718, dec 2005.

[43] D. A. Case, D. S. Cerutti, T. E. Cheatham, III, T. A. Darden, R. E. Duke, H. Giese, H. Gohlke, A.W. Goetz, D. Greene, N. Homeyer, S. Izadi, A. Kovalenko, T. S. Lee, S. LeGrand, P. Li, C. Lin, J. Liu, T. Luchko, R. Luo, D. Mermelstein, K.M. Merz, G. Monard, H. Nguyen, I. Omelyan, A. Onufriev, F. Pan, R. Qi, D.R. Roe, A. Roitberg, C. Sagui, C. L. Simmerling, J. Botello–Smith, J. Swails, R.C. Walker, J. Wang, R. M. Wolf, X. Wu, L. Xiao, D. M. York, and P. A. Kollman. AMBER 2017, 2017.

[44] M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, Ö. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, and D. J. Fox. Gaussian 09, 2013.

[45] 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.

[46] Philip Skehan, Ritsa Storeng, Dominic Scudiero, Anne Monks, James McMahon, David Vistica, Jonathan T. Warren, Heidi Bokesch, Susan Kenney, and Michael R. Boyd. New Colorimetric Cytotoxicity Assay for Anticancer-Drug Screening. *JNCI: Journal of the National Cancer Institute*, 82(13):1107–1112, jul 1990.