Journal of Medicinal Chemistry

Article

pubs.acs.org/jmc

Structure–Activity Studies on the Spiroketal Moiety of a Simplified Analogue of Debromoaplysiatoxin with Antiproliferative Activity

Masayuki Kikumori,[†] Ryo C. Yanagita,[†,‡] Harukuni Tokuda,[§] Nobutaka Suzuki,[§] Hiroshi Nagai,[∥] Kiyotake Suenaga,[⊥] and Kazuhiro Irie*[,†]

[†]Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan

[‡]Department of Applied Biological Science, Faculty of Agriculture, Kagawa University, Kagawa 761-0795, Japan

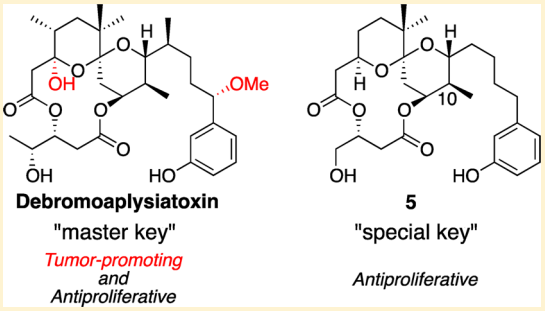
[§]Department of Complementary and Alternative Medicine, Clinical R&D, Graduate School of Medical Science, Kanazawa University, Kanazawa 920-8640, Japan

[∥]Department of Ocean Sciences, Tokyo University of Marine Science and Technology, Tokyo 108-8477, Japan

[⊥]Faculty of Science and Technology, Keio University, Yokohama 223-8522, Japan

Supporting Information

ABSTRACT: Aplog-1, a simplified analogue of tumor-promoting debromoaplysiatoxin, is antiproliferative but not tumor-promoting. Our recent study has suggested that local hydrophobicity around the spiroketal moiety is a crucial determinant for antiproliferative activity. To further clarify the structural features relevant to the activity, we synthesized two methyl derivatives of aplog-1, where a methyl group was installed at position 4 or 10 of the spiroketal moiety. 10-Methyl-aplog-1 (**5**) bound to the C1B domains of novel PKCs (δ, η, and θ) with subnanomolar K_i values, approximately 10–20 times stronger than aplog-1, and markedly inhibited the growth of many human cancer cell lines, while 4-methyl-aplog-1 (**4**) had levels of activity similar to those of aplog-1. Interestingly, **5** showed little tumor-promoting activity unlike the tumor promoter debromoaplysiatoxin. These results suggest that **5** is a potent PKC ligand without tumor-promoting activity and could be a therapeutic lead for the treatment of cancer, like bryostatins.



INTRODUCTION

Tumor promoters are chemicals that enhance tumor formation when applied repeatedly after the initial administration of a small amount of carcinogen.[1] The effects of tumor promoters are mediated mainly by protein kinase C (PKC), a family of serine/threonine kinases that play an important role in cellular signal transduction associated with cell proliferation, differentiation, and apoptosis.[2−4] Potent tumor promoters such as 12-*O*-tetradecanoylphorbol 13-acetate (TPA), teleocidin B-4, and aplysiatoxin (ATX) activate conventional and novel PKC isozymes (PKCs) (Figure 1).[2,5] Conventional PKCs (α, βI, βII, γ) are calcium-dependent, while novel PKCs (δ, ε, η, θ) do not require calcium for their activation. Since PKCs are involved in numerous signal transduction pathways, they are attractive targets in the treatment of cancer[6−9] and other intractable diseases such as diabetes,[10] Alzheimer's disease (AD),[11−13] and acquired immune deficiency syndrome (AIDS).[14] In fact, TPA and artificial PKC activators with a benzolactam skeleton reduced levels of amyloid β, a causative protein for AD.[11,12] TPA is also reported to activate latent human immunodeficiency virus (HIV) and thus represents a possible adjuvant for antivirus therapy.[14] However, its therapeutic use is strictly limited because of adverse effects as a tumor promoter.

Bryostatin 1 (bryo-1)[15] and its congeners[16] isolated from the marine bryozoan *Bugula neritina* are fascinating PKC activators without tumor-promoting activity.[17] Bryo-1 binds to PKCs with subnanomolar K_i values.[18] Since bryo-1 showed significant growth-inhibitory activity against various cancer cell lines, more than 30 clinical trials in phases I and II have been performed against a wide range of tumor types.[6,8,16] Although many of them gave disappointing results for bryo-1 on its own and in combination with other anticancer drugs,[19−24] further investigations are in progress.

In addition to its possible role as an anticancer drug, bryo-1 has therapeutic potential for AD and AIDS where activation of PKCs plays a critical role.[11−14] However, its limited availability from natural sources (18 g of bryo-1 from 13 tons of *B. neritina*)[25] and difficulty in total synthesis[26−28] have hampered further studies on its mode of action and structural optimization as a therapeutic agent. Recently, excellent practical methods for producing bryo-1 and its congeners have been developed by the groups of Wender, Keck, Hale, Trost, and Krische.[29] Wender and colleagues developed simplified analogues of bryo-1 showing more potent antiproliferative

Received: April 21, 2012
Published: May 24, 2012

   dx.doi.org/10.1021/jm300566h | *J. Med. Chem.* 2012, 55, 5614–5626

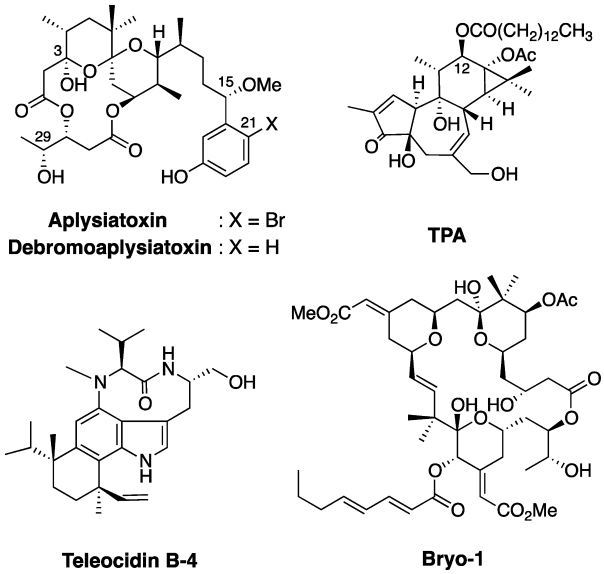


Figure 1. Structure of naturally occurring tumor promoters and bryo-1.

effects than bryo-1.[30,31] Keck and colleagues identified the structural factors responsible for the unique biological activities of bryo-1.[32,33] Trost and colleagues established a practical route for producing bryo-16 as a common intermediate of various bryostatins.[34,35] More recently, Wender, Keck, Hale, and Krische have reported the total synthesis of bryo-9, bryo-1, and bryo-7.[36−39]

In contrast, we identified more synthetically accessible compounds with bryo-1-like activities (aplogs) as another way to address the supply problem.[40−44] Aplog-1 (Figure 2),[40]

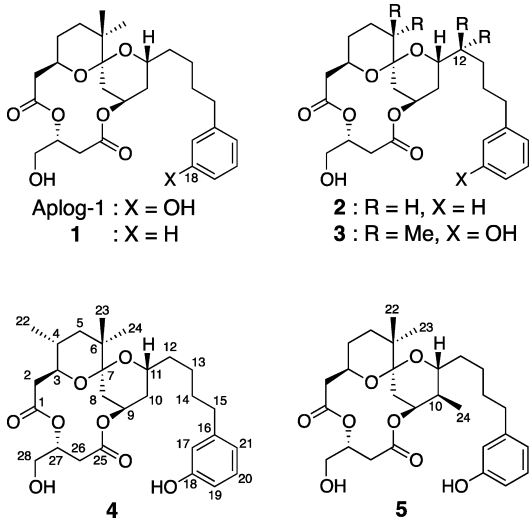


Figure 2. Structure of the simplified analogues of debromoaplysiatoxin.

a simple and less hydrophobic analogue of ATX,[45] is a new PKC ligand with little tumor-promoting activity and showed growth-inhibitory activities against several cancer cell lines with potency comparable to that of bryo-1. In subsequent efforts to identify the structural features relevant to the unique biological profile of aplog-1, we prepared several derivatives altered at positions 6, 12, and 18 (**1**−**3**, Figure 2) and evaluated their antiproliferative activities against 39 human cancer cell lines and binding affinity for PKCδ, which plays a tumor suppressor role and is involved in apoptosis.[40−44] The results clearly showed that the dimethyl groups at position 6 are indispensable to these activities but that the hydroxyl group at position 18 is not necessary.[40,41] Introduction of the dimethyl groups at position 12 did not change the PKC-binding but increased the antiproliferative activities.[43] Of note, the more hydrophobic 12,12-dimethyl-aplog-1 (**3**) did not show any tumor-promoting activity in vitro or in vivo.[43,44] These results suggest that hydrophobicity around the spiroketal moiety of aplog-1 would enhance antiproliferative activities but not tumor-promoting activity.

To further investigate the structure−activity relationship between the antiproliferative and tumor-promoting effects of aplog-1 and to find more effective analogues, two methyl derivatives of aplog-1 (**4**, **5**, Figure 2), in which methyl groups were installed in the spiroketal moiety at positions 4 and 10, were synthesized because these substituents would be responsible for strong binding of ATX to PKCδ. In this article, we describe their antiproliferative activities, tumor-promoting activity in vitro and in vivo, and PKC binding.

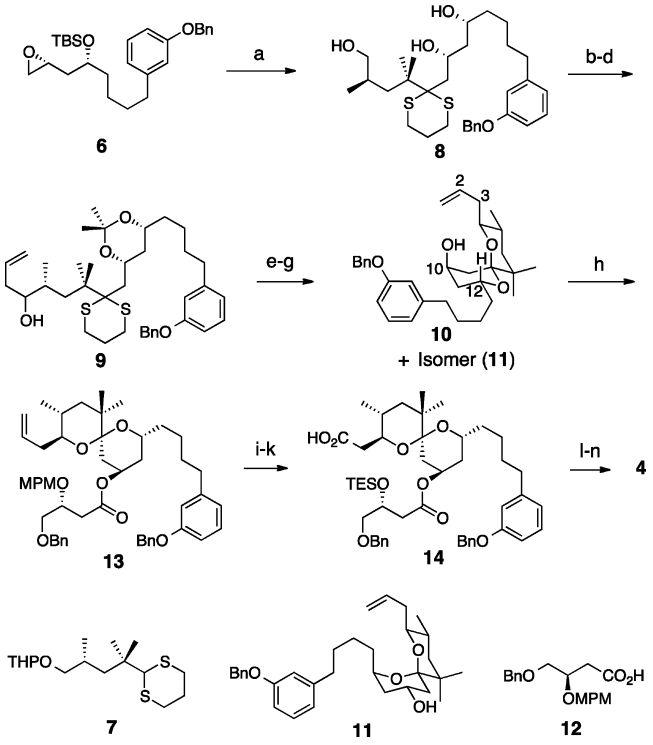
RESULTS AND DISCUSSION

Synthesis of 4 and 5. The 4-methyl derivative of aplog-1 (**4**) was synthesized from a previously reported epoxide (**6**)[40] (Scheme 1). Coupling of **6** with a Kishi's dithiane (**7**)[46] was achieved by the protocol of Ide and Nakata,[47] and methanolysis of the tetrahydropyranyl (THP) ether and *tert*-butyldimethylsilyl (TBS) ether generated a triol (**8**). *O*-Isopropylidenation and oxidation of the primary alcohol provided an aldehyde. Since attempts to install an allyl group streoselectively into the aldehyde employing Keck's[48] or Maruoka's[49] method gave no desired product, probably because of the steric hindrance at the aldehyde group, a Grignard reaction was employed to generate a homoallyl alcohol (**9**) as a 1:1 diastereomeric mixture.

Detachment of the isopropylidene acetal and subsequent hydrolytic cleavage of the dithiane moiety provided three spiroketals (**10**, **11**, and a diastereomer) in a ratio of approximately 1:2:3. The configuration of **10** was determined by nuclear Overhauser enhancement (NOE) experiments (Supporting Information). NOEs were observed between OH-10 and H-2, H-3, or H-12, supporting a desired conformation. Compound **11** proved to be an undesired compound with double axial oxygen atoms. An attempt to convert **11** into **10** employing an acid-catalyzed equilibrium reaction[40] (PPTS, 0.1 equiv, MeOH/DCM = 1:1) gave a disappointing result. In a protic solvent such as MeOH, the undesired spiroketal (**11**) with double anomeric effects could be much more stable than the desired one (**10**) with a single anomeric effect. However, only **10** could form an intramolecular hydrogen bond between the hydroxyl group at position 10 and oxygen of the spiroketal. Since formation of the desired spiroketal (**10**) might be hampered by the intermolecular hydrogen bond between the hydroxyl group at position 10 and the protic solvent, an aprotic solvent (MeCN/DCM = 1:1) along with a stronger acid ((±)-10-camphorsulfonic acid, CSA) were employed. This condition led to a 1:1 mixture of **10** and **11**. No conversion occurred in DCM even if CSA was used as an acid catalyst. Use of PPTS in a mixed solvent (MeCN/DCM = 1:1) also resulted in no conversion. These results indicate both the acidity of the catalyst and permittivity of the solvent to be important in the equilibrium reaction.

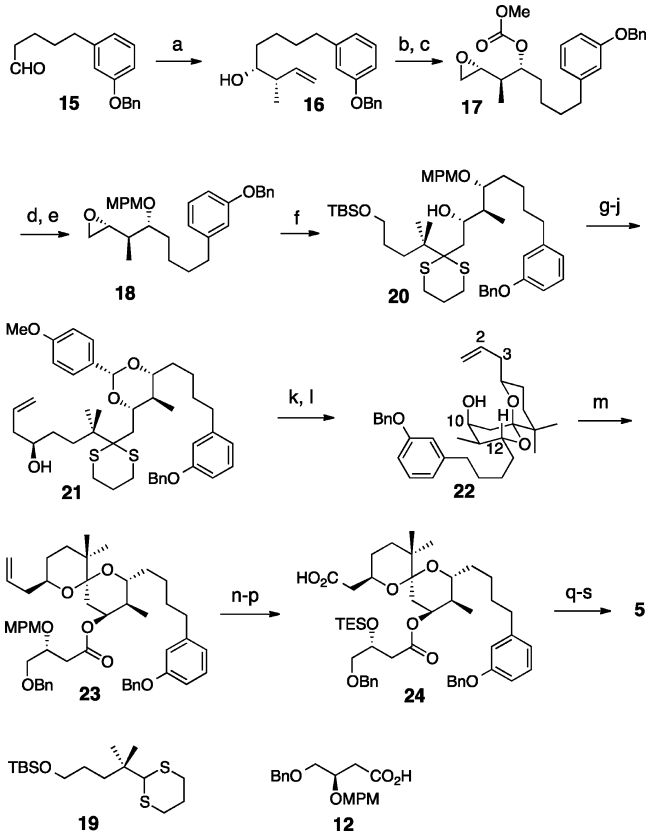
dx.doi.org/10.1021/jm300566h | J. Med. Chem. 2012, 55, 5614−5626

Scheme 1. Synthesis of 4[a]



[a](a) (1) 7, *n*-BuLi, THF; (2) PPTS, MeOH; 83% in two steps; (b) 2,2-dimethoxypropane, CSA, 82%; (c) TPAP, NMO, 4 Å molecular sieves, DCM, 80%; (d) allyl-MgBr, THF, 83%; (e) *p*-TsOH·H_2O, MeCN, THF, H_2O, 95%; (f) $Hg(ClO_4)_2·6H_2O$, DCM, MeCN, H_2O; **10**, 11%; **11**, 23%; (g) CSA, MeCN, DCM; **10**, 41%; **11**, 40%; (h) **12**, TCB-Cl, Et_3N, DMAP, toluene, 94%; (i) DDQ, H_2O, DCM, 94%; (j) TESCl, imidazole, THF, 89%; (k) $KMnO_4$, $NaIO_4$, *t*-BuOH, pH 7 buffer, 59%; (l) HF·pyridine, pyridine, THF, quant; (m) TCB-Cl, Et_3N, DMAP, toluene, 74%; (n) 20% $Pd(OH)_2/C$, H_2, EtOAc, 76%.

Scheme 2. Synthesis of 5[a]



[a](a) *trans*-2-butene, KO-*t*-Bu, *n*-BuLi, (−)-DIP-OMe, $BF_3·Et_2O$, THF, 88%; (b) $(Boc)_2O$, NaHMDS, THF, 87%; (c) (1) IBr, toluene, DCM; (2) K_2CO_3, MeOH; 53% in two steps; (d) NaOMe, MeOH, 73%; (e) MPMCl, NaH, DMF, THF, 97%; (f) **19**, *n*-BuLi, THF, 96%; (g) DDQ, 4 Å molecular sieves, DCM, 87%; (h) PPTS, MeOH, DCM, 82%; (i) TPAP, NMO, 4 Å molecular sieves, DCM, 68%; (j) $TiCl_4$, $Ti(O\text{-}i\text{-}Pr)_4$, Ag_2O, (*S*)-BINOL, allyl-$SnBu_3$, 75%; (k) *p*-TsOH·H_2O, MeCN, THF, H_2O, 41%; (l) $Hg(ClO_4)_2·6H_2O$, DCM, MeCN, H_2O, 62%; (m) **12**, TCB-Cl, Et_3N, DMAP, toluene, 89%; (n) DDQ, H_2O, DCM, 94%; (o) TESCl, imidazole, THF, 95%; (p) $KMnO_4$, $NaIO_4$, *t*-BuOH, pH 7 buffer, 69%; (q) HF·pyridine, pyridine, THF, quant; (r) TCB-Cl, Et_3N, DMAP, toluene, 75%; (s) 10% Pd/C, H_2, EtOH, 78%.

Yamaguchi's esterification[50] of **11** with a known carboxylic acid (**12**),[40] followed by switching of the protecting group from a 4-methoxyphenylmethyl (MPM) group to a triethylsilyl (TES) group, and subsequent oxidative cleavage of the olefin group provided a carboxylic acid (**14**). Deprotection of the TES group, lactonization, and removal of the two benzyl groups gave **4** in 14 steps from **6** with an overall yield of 2.4%.

Synthesis of the 10-methyl derivative of aplog-1 (**5**) started with Brown's diastereoselective crotylation[51] of a known aldehyde (**15**)[40] (Scheme 2) to give a homoallyl alcohol (**16**) with 92% ee (Supporting Information). Stereoselective iodocarbonate cyclization[52] of the homoallyl alcohol generated a cyclic carbonate, which was converted to an epoxide (**17**) by methanolysis. Because of steric hindrance by the methyl group, the methyl carbonate moiety in **17** was a little susceptible to methanolysis with K_2CO_3 at room temperature. To overcome this situation, **17** was subjected to a coupling reaction with **19** according to the same procedure used in the synthesis of **3** reported previously.[43] However, the desired reaction at the epoxide site did not occur possibly because of the instability of the carbonate under the coupling conditions. Hence, we cleaved the methyl carbonate under alkaline conditions. A 2 h treatment of **17** with NaOMe in MeOH at 50 °C gave an alcohol, without formation of a five-membered ether as a byproduct, which was converted to an MPM ether (**18**). Coupling of **18** with a dithiane (**19**) provided **20** in high yield (96%).

Oxidative acetal formation and deprotection of the TBS ether provided a primary alcohol, the oxidation of which, followed by Maruoka's asymmetric allylation,[49] produced a homoallyl alcohol (**21**). Because of the high stability of the *p*-methoxybenzylidene acetal moiety, acid hydrolysis of **21** gave a triol in moderate yield (41%). Unlike the synthesis of **4**, hydrolysis of the dithiane moiety of the triol with $Hg(ClO_4)_2·6H_2O$ produced the desired isomer **22** as a main product (62%) because of the 1,3-diaxal interaction between the hydrogen atom at position 9 and the methyl group at position 11 in the undesired isomer. Subsequent construction of the diolide ring was achieved by procedures similar to those used for the synthesis of **4**. Compound **5** was obtained in 20 steps from **15** with an overall yield of 0.85%.

Antiproliferative Activities against 39 Human Cancer Cell Lines. Antiproliferative activities of **4** and **5** were initially evaluated using a panel of 39 human cancer cell lines as described previously.[53] The growth inhibitory activity was

dx.doi.org/10.1021/jm300566h | *J. Med. Chem.* 2012, 55, 5614−5626

expressed as a GI_{50} (M), the concentration required to inhibit cell growth by 50% compared to an untreated control (Experimental Section). The average of the log GI_{50} values for all 39 human cancer cell lines was expressed as a MG-MID. Since the MG-MID of aplog-1 was −4.97, the cell lines with log GI_{50} values less than −5.00 except for SNB-78 are listed in Table 1 (the rest of the data are provided in the Supporting Information). The MG-MIDs of 4-methyl-aplog-1 (**4**) and 10-methyl-aplog-1 (**5**) were −5.03 and −5.24, respectively. Interestingly, tumor-promoting debromoaplysiatoxin (DAT) also showed antiproliferative activity; the MG-MID was −5.22.

Table 1. Growth Inhibition of Debromoaplysiatoxin (DAT), Aplog-1, 4, and 5 against Several Human Cancer Cell Lines

		log GI_{50} (log *M*)			
cancer type	cell line	DAT	aplog-1[a]	**4**	**5**
breast	HBC-4	−6.47	−6.33	−6.56	−7.48
	MDA-MB-231	−6.03	−5.61	−5.81	−6.90
CNS	SNB-78	−4.80	−4.72	−4.76	−6.05
colon	HCC2998	−6.09	−5.43	−5.39	−6.47
lung	NCI-H460	−6.46	−5.60	−5.90	−7.07
	A549	−5.94	−5.32	−5.13	−6.01
melanoma	LOX-IMVI	−5.69	−5.74	−5.38	−6.21
stomach	St-4	−6.44	−5.55	−5.22	−6.24

[a]Cited from ref 40.

As shown in Table 1, the growth inhibitory activities of aplog-1 and 4-methyl-aplog-1 (**4**) were similar. On the other hand, 10-methyl-aplog-1 (**5**) exhibited a 5−20 times stronger inhibitory effect on the growth of the cancer cells listed in Table 1 than aplog-1. Moreover, the antiproliferative activities of **5** were greater than those of DAT except for St-4. To examine the relationship between the MG-MID and molecular hydrophobicity, log *P* values of these compounds were evaluated by a method using HPLC[54] (Table 2). The hydrophobicity of **4** and **5** was similar and slightly higher than that of aplog-1. As expected, DAT was the most hydrophobic of these compounds. These results indicate that the antiproliferative activities of aplogs do not simply depend on molecular hydrophobicity and that the local hydrophobicity around position 10 would play an important role in enhancing antiproliferative activities.

Table 2. Values of log *P* of Debromoaplysiatoxin (DAT), Aplog-1, 4, and 5, Determined by HPLC[54]

	DAT	aplog-1	**4**	**5**
log *P*	4.4	3.3	3.7	3.6

Tumor-Promoting Activity. The most likely adverse effect of aplog-1 and its derivatives (**4** and **5**) would be tumor promotion because these compounds possess the skeleton of tumor-promoting ATX and DAT. Thus, we evaluated the tumor-promoting activities of **4** and **5** by testing induction of Epstein−Barr virus early antigen (EBV-EA).[55,56] EBVs are under the strict control of the host human B lymphoblastoid Raji cells. They are activated when treated with chemicals such as tumor promoters and produce early antigen, which can be detected by an indirect immunofluorescence technique. The EBV-EA-inducing activities are expressed as the percentage of EA-positive cells. Compared to DAT that showed significant EA induction like TPA, both **4** and **5** exhibited only weak EA induction (<10%) even at 1 μM, like aplog-1 (Figure 3), suggesting that both methyl groups at positions 4 and 10 are not crucial for tumor-promoting activity.

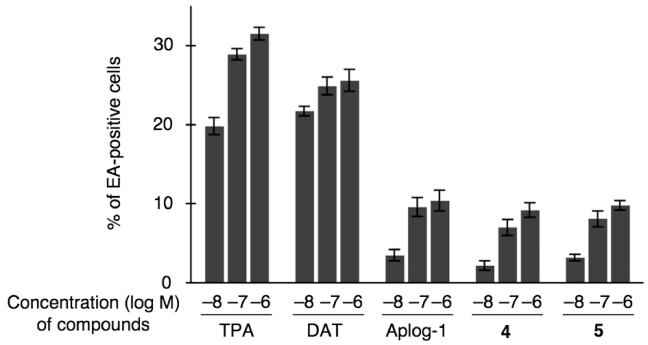


Figure 3. EBV-EA production induced by TPA, debromoaplysiatoxin (DAT), aplog-1, **4**, and **5**. Percentages of EA-positive cells are shown. Sodium *n*-butyrate (4 mM) was added to all samples to enhance the sensitivity of the Raji cells. Only 0.1% of the cells were positive for EA at 4 mM sodium *n*-butyrate. The final concentration of DMSO was 0.4%. Cell viability exceeded 60% in all experiments except for that with TPA at 10^{-6} M (>50%). Error bars represent standard error of the mean (n = 3).

Since **5** exhibited more potent antiproliferative activities than **4** and aplog-1, the in vivo tumor-promoting activity was evaluated by a two-stage carcinogenesis experiment on mouse skin (Figure 4). The skin on the back of ICR mice was treated with a single dose of 390 nmol of 7,12-dimethylbenz[*a*]-

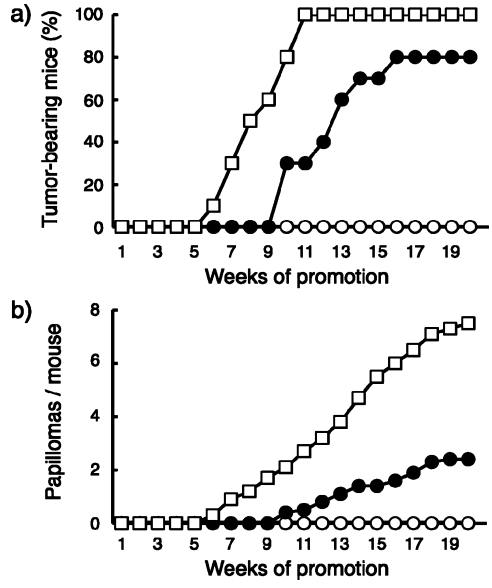


Figure 4. Tumor-promoting activity of TPA, debromoaplysiatoxin (DAT), and **5**. The back of each male 6-week-old ICR mice was shaved with surgical clippers. From a week after initiation by a single application of 390 nmol of DMBA in 0.1 mL acetone, 8.5 nmol of **5** in 0.1 mL of acetone was applied twice a week from week 1 to week 20 (○). The control group was treated with DMBA and 1.7 nmol TPA (□) or DAT (●). Each group consisted of 10 mice. (a) Tumor-bearing mice (%). The differences between TPA and DAT at weeks 8, 9, 11, and 12 were statistically significant ($p < 0.05$, Fisher's exact test). (b) Number of papillomas per mouse. The differences between TPA and DAT at weeks 9−20 were statistically significant ($p < 0.05$, Welch's *t*-test).

dx.doi.org/10.1021/jm300566h | J. Med. Chem. 2012, 55, 5614−5626

anthracene (DMBA) and from 1 week later, with 8.5 nmol of **5** (5-fold excess of TPA and DAT) twice a week. Compound **5** did not induce any tumors at week 20. In a control experiment using TPA (1.7 nmol twice a week), the first tumor appeared in week 6 and the proportion of tumor-bearing mice reached 100% at week 11. The number of papillomas/mice was 8.0 in week 20. Moreover, DAT (1.7 nmol twice a week) also generated tumors, albeit weakly compared with TPA. DAT's weak tumor-promoting activity coincided with that reported previously.[57]

According to Suganuma and colleagues, oscillatoxin A, which lacks DAT's methyl group at position 29, exhibited tumor-promoting activity in vitro, suggesting that this methyl group is not important for tumor-promoting activity.[57] Nakamura and colleagues reported that 3-deoxy-DAT and DAT are equipotent as PKC activators.[58] However, the tumor-promoting activity of 3-deoxy-DAT remains unknown. Taken together, the hemiacetal hydroxy group at position 3 and/or the methoxy group at position 15 in DAT would be responsible for the tumor-promoting activity.

Binding Affinity for PKCs. The activation of PKCs is likely to be responsible for the antiproliferative and tumor-promoting activities of ATX-related compounds. We evaluated the affinity of DAT and the methyl derivatives of aplog-1 (**4**, **5**) for C1 domains (C1A and C1B) of PKCs (Table 3). The C1A peptides were used as conventional PKC surrogates, and the C1B peptides were employed as novel PKC surrogates, since these domains are the main binding sites of tumor promoters.[59−62] Our accumulated data suggest that the binding assay using PKC C1 peptides is a reliable system for evaluating binding affinities of tumor promoters for PKC isozymes. The binding constants of the C1 peptides for tumor promoters such as phorbol 12,13-dibutyrate (PDBu)[59] and (−)-indolactam-V[63] (Supporting Information) were almost equivalent to those of corresponding whole PKC isozymes obtained by the well established procedure of Sharkey and Blumberg.[64] Thus, assessment of the binding affinities and selectivity for PKC isozymes can be realized by a simple procedure using PKC C1 peptides. Recently, we reported that tumor promoters like PDBu and ATX bound potently to the C1 domains of both conventional and novel PKCs, while antiproliferative compounds such as aplog-1 and bryo-1 exhibited some selectivity for novel PKCs other than PKCε, that is, PKCδ, PKCη, and PKCθ.[44] The affinity of DAT for the PKC C1 domains was quite similar to that of ATX as reported previously.[41]

Table 3. Values of K_i for the Inhibition of [^{3}H]PDBu Binding by Debromoaplysiatoxin (DAT), Aplog-1, 4, and 5

	K_i (nM)			
PKC C1 peptide	DAT[a]	aplog-1[b]	**4**[a]	**5**[a]
α-C1A	0.26(0.08)	63	15(2)	4.7(0.7)
β-C1A	0.17(0.02)	89	15(2)	12(0.5)
γ-C1A	0.38(0.06)	39	12(2)	5.5(0.4)
δ-C1B	0.20(0.01)	7.4	3.3(0.3)	0.46(0.06)
ε-C1B	0.63(0.08)	25	17(2)	2.0(0.2)
η-C1B	0.11(0.02)	4.4	5.9(0.5)	0.45(0.05)
θ-C1B	0.11(0.03)	4.0	2.9(0.2)	0.54(0.08)

[a]Values in parentheses represent the standard deviation from at least three separate experiments. [b]Cited from ref 40.

Although the affinity of **4** for PKCδ-C1B was 2 times stronger than that of aplog-1, the binding selectivity of **4** for novel PKC-C1B domains was relatively poor. On the other hand, **5** displayed stronger affinity, 1 order of magnitude, for all PKC C1 peptides than aplog-1; the K_i of **5** for PKCδ-C1B was comparable to that of DAT and bryo-1 (K_i = 0.60 nM),[65] and **5** showed some selectivity for novel PKC-C1B domains like aplog-1. These results suggest that introduction of a methyl group into position 4 could enhance the affinity for conventional PKCs rather than for novel PKCs and that introduction of a methyl group into position 10 would enhance the affinity for both conventional and novel PKCs. These results may also imply binding for conventional PKCs to be linked to tumor-promoting activity rather than antiproliferative activities. In fact, activation of PKCα was suggested to be involved in cancer cell growth,[66,67] and PKCδ to play a tumor suppressor role and to be involved in apoptosis.[68,69]

Since a docking model for ATX and the C1B domain of PKCδ has not yet been proposed, it is not easy to explain the affinity of **5** for PKCδ. However, overlapping of the pharmacophores of ATX and TPA was reported,[70] which indicated that the spiroketal moiety of ATX occupies positions in space similar to the C-12 residues of TPA. The C-12 region of phorbol esters was located on a hydrophobic surface of the crystal structure of the PKCδ C1B domain[71] and would be involved in hydrophobic interaction with the C1B domain and phosphatidylserine in cellular membranes.[72] Moreover, methyl groups at positions 4 and 10 do not seem to affect significantly the conformation of the spiroketal moiety and the macrolactone ring, since each spiroketal intermediate (**10**, **22**) showed an NOE pattern similar to that of aplog-1 (Supporting Information),[40] and ^{1}H NMR coupling constants of the diolide moiety of **4** and **5** were also similar to those of aplog-1.[40] Taken together, the methyl groups at positions 4 and 10 of ATX might be involved in hydrophobic interaction, analogous to TPA, and the methyl group at position 10 might interact specifically with the hydrophobic residues of novel PKCs and/or have a role in orienting properly the adjacent side chain at position 11.

CONCLUSION

Our previous studies indicated that the spiroketal moiety of aplog-1 was deeply involved in PKC binding and antiproliferative activities against several cancer cell lines. To further study the structure−activity relationship, we synthesized 4-methyl- and 10-methyl-aplog-1 (**4**, **5**). To our delight, the introduction of a methyl group at position 10 enhanced the affinity for PKCδ but not tumor-promoting activity in vitro as well as in vivo. Compound **5** had the strongest antiproliferative effects against cancer cell lines of any aplog synthesized to date (aplog-1, **1**−**4**). The compound could thus be a new drug lead for cancer, like bryo-1. It is remarkable that the antiproliferative activities of ATX-related compounds did not simply depend on molecular hydrophobicity but correlated with the specific interaction with the C1 domain of PKCs. PKC ligands that bind more strongly and more selectively to PKCδ might become a more pertinent lead in the development of drugs for treating cancer.

DAT still retained, albeit weak, tumor-promoting activity in vivo but inhibited the growth of cancer cells more potently than aplog-1 (Table 1). On the other hand, **5** showed little tumor-promoting activity and exhibited stronger antiproliferative activities than DAT. These observations suggest that DAT is a "master key" with tumor-promoting and antiproliferative activities whereas its simplified analogue **5** is a "special key" with only antiproliferative activities. Such simplification is quite

important in developing new medicinal leads from natural products. Further studies are necessary to identify the structural factors of DAT involved in the tumor-promoting activity.

EXPERIMENTAL SECTION

General Remarks. The following spectroscopic and analytical instruments were used: for UV, UV-2200A (Shimadzu, Kyoto, Japan); digital polarimeter, DIP-1000 (Jasco, Tokyo, Japan); ^{1}H and ^{13}C NMR, Avance I 400 and Avance III 500 (reference TMS, Bruker, Germany); HPLC, model 600E with a model 2487 UV detector (Waters, Tokyo, Japan); HR-FAB-MS, JMS-600H (JEOL, Tokyo, Japan) and JMS-700 (JEOL, Tokyo, Japan). HPLC was carried out on YMC packed ODS-A AA12S05-1520WT and SIL SL12S05-2510WT (Yamamura Chemical Laboratory, Kyoto, Japan) and CHIRALCEL OJ-RH (Daicel Corporation, Osaka, Japan). Wakogel C-200 (silica gel, Wako Pure Chemical Industries, Osaka, Japan) and YMC A60-350/250 gel (ODS, Yamamura Chemical Laboratory, Kyoto, Japan) were used for column chromatography. [^{3}H]PDBu (22.1 Ci/mmol) was purchased from Perkin-Elmer Life Sciences Research Products (Boston, MA, U.S.). The PKC C1 peptides were synthesized as reported previously.[73] All other chemicals and reagents were purchased from chemical companies and used without further purification. The purity of the final compounds was greater than 95% as judged by HPLC (for details, see Supporting Information).

Synthesis of 8. To a solution of 7 (1.88 g, 5.68 mmol, 2 equiv) in dry THF (27.5 mL) was added 1.6 M *n*-BuLi in hexane (3.9 mL) at room temperature under an Ar atmosphere. After 45 min of stirring at room temperature, the mixture was cooled to 4 °C. A solution of **6** (1.25 g, 2.85 mmol) in dry THF (15 mL) was then added, and the reaction mixture was stirred for 2 h at 4 °C. The reaction was quenched with saturated aqueous NH_4Cl (25 mL). The mixture was poured into EtOAc (50 mL) and H_2O (50 mL). After the organic layer was separated, the aqueous layer was extracted with EtOAc (20 mL × 2). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, 5% → 10% EtOAc/hexane) to afford a coupling product (2.03 g) as a clear oil. To a solution of the coupling product (2.03 g) in MeOH (60 mL) was added PPTS (1.34 g, 5.34 mmol, 2 equiv) at room temperature. After 17 h of stirring, the reaction mixture was concentrated in vacuo. The residue was poured into EtOAc (20 mL) and H_2O (20 mL). After the organic layer was separated, the aqueous layer was extracted with EtOAc (20 mL × 2). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, 40% → 70% EtOAc/hexane) to afford **8** (1.32 g, 2.36 mmol, 88% in two steps) as a clear oil. ^{1}H NMR (400 MHz, 297.8 K, $CDCl_3$, 0.023 M) δ 1.00 (3H, d, J = 6.7 Hz), 1.15 (3H, s), 1.18 (3H, s), 1.35−1.72 (11H, m), 1.90−1.97 (3H, m), 2.17 (1H, dd, J = 15.5, 8.9 Hz), 2.60 (2H, t, J = 7.7 Hz), 2.81−3.00 (4H, m), 3.40 (1H, dd, J = 10.4, 6.6 Hz), 3.46 (1H, dd, J = 10.4, 6.0 Hz), 3.87 (1H, m), 4.52 (1H, m), 5.05 (2H, s), 6.78−6.82 (3H, m), 7.19 (1H, t, J = 7.8 Hz), 7.30−7.45 (5H, m) ppm. ^{13}C NMR (100 MHz, 298.6 K, $CDCl_3$, 0.035 M) δ 19.80, 22.49, 22.91, 25.15, 27.33, 27.52, 31.43, 32.11, 36.00, 37.60, 39.48, 44.36, 45.04, 46.31, 63.24, 69.35, 69.91, 71.22, 71.72, 77.22, 111.79, 115.15, 121.16, 127.54 (2C), 127.89, 128.55 (2C), 129.21, 137.20, 144.46, 158.85 ppm. HR-FAB-MS (matrix, *m*-nitrobenzyl alcohol): m/z = 561.3074 ([MH]$^+$, calcd for $C_{32}H_{49}O_4S_2$ 561.3072). $[\alpha]^{24.7}_D$ +9.2° (c 0.34, $CHCl_3$).

Synthesis of 9. To a solution of **8** (1.32 g, 2.36 mmol) in DCM (20 mL) were added 2,2-dimethoxypropane (2.89 mL, 23.6 mmol, 10 equiv) and (±)-10-camphorsulfonic acid (110 mg, 0.472 mmol, 0.2 equiv) at room temperature. The mixture was stirred at room temperature for 30 min, and the reaction was quenched with saturated aqueous $NaHCO_3$ (20 mL). The organic layer was separated, and the aqueous layer was extracted with DCM (20 mL × 2). The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was dissolved in DCM (20 mL), and silica gel (2.0 g) was added. After the mixture was stirred at room temperature for 30 min, the silica gel was removed by filtration. The filtrate was concentrated in vacuo, and the residue was purified by column chromatography (silica gel, 10% → 30% EtOAc/hexane) to afford an acetonide (1.16 g, 1.93 mmol, 82%) as a clear oil. To a solution of the acetonide (1.16 g, 1.93 mmol), NMO (350 mg, 2.90 mmol, 1.5 equiv), and 4 Å molecular sieves (966 mg) in DCM (16 mL) was added a suspension of tetrapropylammonium perruthenate (34 mg, 0.097 mmol, 0.05 equiv) in DCM (2 mL). After 15 min of stirring at room temperature, the reaction mixture was absorbed in a small amount of silica gel. The resulting gel was directly loaded onto a silica gel column and eluted with 10% EtOAc/hexane. Concentration of the fractions gave an aldehyde (922 mg, 1.54 mmol, 80%) as a clear oil. To a solution of the aldehyde (384 mg, 0.643 mmol) in THF was added 1 M solution of allyl-MgBr in Et_2O (965 μL) at −78 °C under an Ar atmosphere. After 30 min of stirring at the same temperature, the reaction mixture was warmed to room temperature. The reaction mixture was quenched with saturated aqueous NH_4Cl (10 mL). After the organic layer was separated, the aqueous layer was extracted with EtOAc (10 mL × 2). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, 10% → 20% EtOAc/hexane) to afford **9** (340 mg, 0.532 mmol, 83%) as a clear oil and a 1:1 diastereomeric mixture. ^{1}H NMR (400 MHz, 297.8 K, $CDCl_3$, 0.087 M) δ 0.97 (3H, d, J = 5.4 Hz), 0.99 (3H, d, J = 5.4 Hz), 1.14 (3H, s), 1.15 (3H, s), 1.17 (3H, s), 1.18 (3H, s), 1.22−1.67 (22H, m), 1.37 (6H, s), 1.42 (6H, s), 1.75−2.39 (14H, m), 2.59 (4H, t, J = 7.7 Hz), 2.65−2.73 (4H, m), 2.80−2.87 (2H, m), 3.02−3.10 (2H, m), 3.45 (1H, m), 3.52 (1H, m), 3.81 (2H, br s), 4.22 (1H, br s), 4.25 (1H, br s), 5.05 (4H, s), 5.11−5.17 (4H, m), 5.78−5.90 (2H, m), 6.77−6.81 (6H, m), 7.19 (2H, t, J = 7.8 Hz), 7.30−7.45 (10H, m) ppm. ^{13}C NMR (100 MHz, 298.3 K, $CDCl_3$, 0.087 M) δ 17.12 (2C), 18.55 (2C), 19.65 (2C), 22.58 (2C), 23.18 (2C), 24.67, 24.78, 26.96, 27.04, 30.34 (2C), 31.31 (2C), 33.95 (2C), 34.44 (2C), 35.89 (2C), 36.31 (2C), 37.89 (2C), 38.66 (2C), 39.07 (2C), 43.59 (2C), 44.31, 44.44, 63.64, 63.68, 68.04, 68.08, 69.15 (2C), 69.91 (2C), 75.51, 75.61, 98.57 (2C), 111.73 (2C), 115.20 (2C), 117.79, 118.01, 121.13 (2C), 127.51 (4C), 127.91 (2C), 128.56 (4C), 129.21 (2C), 135.66, 135.68, 137.15 (2C), 144.38 (2C), 158.86 (2C) ppm. HR-FAB-MS (matrix, *m*-nitrobenzyl alcohol): m/z = 663.3511 ([M + Na]$^+$, calcd for $C_{38}H_{56}O_4S_2Na$ 663.3518).

Synthesis of 10. To a solution of **9** (640 mg, 1.00 mmol) in MeCN (17 mL) and THF (1.7 mL) were added H_2O (1.7 mL) and *p*-TsOH·H_2O (571 mg, 3.00 mmol, 3.0 equiv) at room temperature. The mixture was stirred at room temperature for 2 h, and then the reaction was quenched with saturated aqueous $NaHCO_3$ (20 mL). The mixture was extracted with EtOAc (30 mL × 3). The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, 30% → 50% EtOAc/hexane) to afford a triol (568 mg, 0.947 mmol, 95%) as a clear oil. To a solution of the triol (568 mg, 0.947 mmol) in MeCN (1.26 mL), DCM (1.26 mL), and H_2O (1.26 mL) was added $Hg(ClO_4)_2{\cdot}6H_2O$ (387 mg, 0.762 mmol, 2.0 equiv) at 4 °C. After 1 h of stirring at the same temperature, the reaction mixture was poured into EtOAc (30 mL) and saturated aqueous $Na_2S_2O_3$ (30 mL). The organic layer was separated, and the aqueous layer was extracted with EtOAc (30 mL × 2). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by column chromatography (5% → 10% → 20% EtOAc/hexane) to afford a mixture containing **10** (105 mg) and **11** (43.0 mg, 0.087 mmol, 23%). The former was further purified by HPLC (column, YMC-Pack ODS-A AA12S05-1520WT; solvent, 85% MeCN/H_2O; flow rate, 8.0 mL/min; pressure, 900 psi; UV detector, 254 nm; retention time, 50 min) to afford **10** (20.5 mg, 0.042 mmol, 11%). Compound **10**: ^{1}H NMR (400 MHz, 297.8 K, $CDCl_3$, 0.069 M) δ 0.82 (3H, d, J = 6.5 Hz), 0.84 (3H, s), 0.98 (3H, s), 1.25−1.73 (11H, m), 1.51 (1H, dd, J = 14.4, 3.4 Hz), 2.16 (1H, dt, J = 13.9, 8.6 Hz), 2.28 (1H, ddd, J = 14.4, 2.7, 1.9 Hz), 2.42 (1H, m), 2.59 (2H, t, J = 7.7 Hz), 3.36 (1H, ddd, J = 10.3, 8.6, 2.6 Hz), 3.63 (1H, d, J = 11.5 Hz, OH), 4.06 (1H, m), 4.14 (1H, m), 5.05 (2H, s), 5.12−5.16 (2H, m), 5.90 (1H, m), 6.78−6.83 (3H, m), 7.19 (1H, t, J = 7.8 Hz), 7.30−7.45 (5H, m) ppm. ^{13}C NMR (100

dx.doi.org/10.1021/jm300566h | J. Med. Chem. 2012, 55, 5614−5626

MHz, 298.6 K, $CDCl_3$, 0.069 M) δ 17.78, 22.01, 24.90, 25.91, 27.98, 30.78, 31.15, 35.73, 36.02, 37.80, 38.13, 38.35, 44.10, 63.69, 65.78, 69.89, 78.42, 102.21, 111.71, 115.15, 118.34, 121.15, 127.51 (2C), 127.87, 128.53 (2C), 129.16, 134.87, 137.22, 144.57, 158.85 ppm. HR-FAB-MS (matrix, *m*-nitrobenzyl alcohol): m/z = 493.3288 ($[MH]^+$, calcd for $C_{32}H_{45}O_4$ 493.3318). $[\alpha]^{10.3}_D$ +40.6° (*c* 0.42, $CHCl_3$). Compound **11**: ^{1}H NMR (400 MHz, 297.9 K, $CDCl_3$, 0.153 M) δ 0.76 (3H, d, J = 5.9 Hz), 0.85 (3H, s), 0.96 (3H, s), 1.03 (1H, m), 1.22−1.67 (11H, m), 1.95 (1H, m), 2.07 (2H, m), 2.29 (1H, m), 2.59 (2H, t, J = 7.6 Hz), 3.42 (1H, m), 3.88 (1H, m), 4.21 (1H, m), 4.97−5.03 (2H, m), 5.04 (2H, s), 5.87 (1H, m), 6.78−6.82 (3H, m), 7.19 (1H, t, J = 7.8 Hz), 7.30−7.45 (5H, m) ppm. ^{13}C NMR (100 MHz, 298.8 K, $CDCl_3$, 0.153 M) δ 17.86, 23.69, 25.58, 26.11, 30.62, 31.24, 35.54, 35.99, 36.29, 37.52, 37.59, 37.82, 42.87, 62.43, 69.90, 71.64, 74.56, 101.88, 111.76, 115.19, 115.90, 121.15, 127.51 (2C), 127.89, 128.55 (2C), 129.21, 136.06, 137.18, 144.35, 158.85 ppm. HR-FAB-MS (matrix, *m*-nitrobenzyl alcohol): m/z = 493.3303 ($[MH]^+$, calcd for $C_{32}H_{45}O_4$ 493.3318). $[\alpha]^{11.7}_D$ +6.4° (*c* 0.58, $CHCl_3$).

To a solution of **11** (21 mg, 0.043 mmol) in MeCN (0.6 mL) and DCM (0.6 mL) was added (±)-10-camphorsulfonic acid (1.0 mg, 4.3 μmol, 0.1 equiv) at room temperature. After 1 h of stirring at room temperature, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ (3 mL). The mixture was extracted with EtOAc (3 mL × 3). The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, 5% → 10% EtOAc/hexane) to afford **10** (8.7 mg, 0.018 mmol, 41%) and **11** (8.5 mg, 0.017 mmol, 40%).

Synthesis of 13. To a solution of **12** (110 mg, 0.335 mmol, 1.36 equiv) and Et_3N (53 μL, 0.379 mmol, 1.53 equiv) in toluene (2.5 mL) was added 2,4,6-trichlorobenzoyl chloride (43.0 μL, 0.275 mmol, 1.7 equiv) at room temperature. After 2 h of stirring at room temperature, a supernatant of the resulting suspension was added to a solution of **10** (122 mg, 0.247 mmol) and DMAP (57 mg, 0.379 mmol, 1.89 equiv) in toluene (4.5 mL) at 50 °C. The resulting mixture was stirred at the same temperature for 1 h and then poured into water (10 mL). The mixture was extracted with EtOAc (20 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% → 10% EtOAc/hexane) to afford **13** (186 mg, 0.231 mmol, 94%) as a clear oil. ^{1}H NMR (400 MHz, 297.3 K, $CDCl_3$, 0.032 M) δ 0.74 (3H, d, J = 6.5 Hz), 0.84 (3H, s), 0.97 (3H, s), 1.17 (1H, t, J = 13.5 Hz), 1.28 (1H, dd, J = 13.5, 4.7 Hz), 1.32−1.47 (5H, m), 1.53−1.69 (5H, m), 2.15−2.24 (2H, m), 2.36 (1H, m), 2.56 (2H, t, J = 7.7 Hz), 2.60 (2H, m), 3.05 (1H, m), 3.50 (1H, dd, J = 10.0, 3.5 Hz), 3.58 (1H, dd, J = 10.0, 5.3 Hz), 3.78 (3H, s), 4.08 (1H, m), 4.20 (1H, m), 4.52 (1H, d, J = 11.2 Hz), 4.53 (2H, s), 4.59 (1H, d, J = 11.2 Hz), 4.98−5.03 (2H, m), 5.04 (2H, s), 5.10 (1H, m), 6.00 (1H, m), 6.77−6.86 (5H, m), 7.18 (1H, t, J = 7.8 Hz), 7.21−7.45 (12H, m) ppm. ^{13}C NMR (100 MHz, 297.7 K, $CDCl_3$, 0.032 M) δ 17.39, 21.77, 24.90, 25.68, 26.10, 29.93, 31.11, 34.81, 35.53, 36.09, 37.92, 38.07, 38.18, 44.28, 55.25, 63.90, 68.31, 69.87, 71.88, 72.13, 73.33, 74.81, 77.22, 99.73, 111.72, 113.73 (2C), 115.10, 116.24, 121.16, 127.53 (2C), 127.58 (2C), 127.62, 127.86, 128.38 (2C), 128.53 (2C), 129.14, 129.36 (2C), 130.60, 135.44, 137.25, 138.17, 144.67, 158.83, 159.19, 171.61 ppm. HR-FAB-MS (matrix, *m*-nitrobenzyl alcohol): m/z = 827.4532 ($[M + Na]^+$, calcd for $C_{51}H_{64}O_8Na$ 827.4499). $[\alpha]^{26.5}_D$ +11.3° (*c* 1.17, $CHCl_3$).

Synthesis of 14. To a vigorously stirred solution of **13** (179 mg, 0.223 mmol) in DCM (12 mL) and H_2O (2.0 mL) was added DDQ (104 mg, 0.445 mmol, 2.0 equiv) at room temperature, causing a brown color to appear. After 1 h of stirring at room temperature, the color gradually changed to yellow-orange with precipitation of a solid. The mixture was poured into saturated aqueous $NaHCO_3$ (30 mL) and extracted with DCM (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, 10% → 30% EtOAc/hexane) to afford an alcohol (143 mg, 0.209 mmol, 94%) as a clear oil. To a solution of the alcohol (139 mg, 0.203 mmol) in THF (1.6 mL) were added imidazole (41 mg, 0.609 mmol, 3.0 equiv) and chlorotriethylsilane (60 μL, 0.359 mmol, 1.8 equiv) at room temperature. After the mixture was stirred for 1 h at room temperature, the reaction was quenched with brine (5 mL). The resulting mixture was extracted with EtOAc (10 mL × 3). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, 2.5% → 5%) to afford a silyl ether (145 mg, 0.182 mmol, 89%) as a clear oil. To a suspension of $NaIO_4$ (103 mg, 0.482 mmol, 8.0 equiv) in pH 7.2 phosphate buffer (4.8 mL) was added $KMnO_4$ (9.5 mg, 0.0603 mmol, 1.0 equiv) in one portion. After 10 min of stirring at room temperature under an Ar atmosphere, the mixture was added to a solution of the silyl ether (48.1 mg, 0.0603 mmol) in *t*-BuOH (4.8 mL). The reaction mixture was stirred at room temperature for 17 h, and the reaction was quenched with $Na_2S_2O_3$ (28.9 mg). The resulting mixture was poured into EtOAc (20 mL) and water (20 mL). The organic layer was separated, and the aqueous layer was extracted with EtOAc (20 mL × 3). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, 10% → 20% EtOAc/hexane containing 0.1% AcOH) to afford **14** (29.2 mg, 0.0358 mmol, 59%) as a clear oil. ^{1}H NMR (500 MHz, 295.3 K, $CDCl_3$, 0.025 M) δ 0.58 (6H, q, J = 7.9 Hz), 0.82 (3H, d, J = 6.6 Hz), 0.88 (3H, s), 0.91 (9H, t, J = 7.9 Hz), 1.01 (3H, s), 1.20−1.30 (2H, m), 1.31−1.80 (9H, m), 1.71 (1H, dd, J = 15.3, 4.4 Hz), 2.18 (1H, br d, J = 15.3 Hz), 2.48−2.64 (5H, m), 2.67 (1H, dd, J = 15.2, 3.6 Hz), 3.38 (1H, dd, J = 9.5, 6.6 Hz), 3.49 (1H, dd, J = 9.5, 5.0 Hz), 3.50 (1H, m), 4.10 (1H, m), 4.31 (1H, m), 4.51 (1H, d, J = 12.1 Hz), 4.54 (1H, d, J = 12.1 Hz), 5.05 (2H, s), 5.10−5.11 (1H, m), 6.78−6.83 (3H, m), 7.18 (1H, t, J = 7.8 Hz), 7.27−7.45 (10H, m) ppm. ^{13}C NMR (125 MHz, 295.8 K, $CDCl_3$, 0.025 M) δ 4.88 (3C), 6.77 (3C), 17.52, 22.04, 24.96, 25.93, 26.61, 30.84, 31.19, 34.45, 35.56, 35.99, 38.18, 38.82, 40.60, 43.65, 65.03, 67.58, 68.49, 69.96, 73.44, 74.07, 75.55, 101.58, 111.88, 115.19, 121.22, 127.54 (2C), 127.76, 127.81 (2C), 127.86, 128.41 (2C), 128.54 (2C), 129.20, 137.29, 137.91, 144.45, 158.90, 171.57, 171.65 ppm. HR-FAB-MS (matrix, *m*-nitrobenzyl alcohol): m/z = 839.4557 ($[M + Na]^+$, calcd for $C_{48}H_{68}O_9SiNa$ 839.4531). $[\alpha]^{28.6}_D$ +16.5° (*c* 1.00, $CHCl_3$).

Synthesis of 4. To a solution of **14** (29.2 mg, 0.0358 mmol) in THF (3.0 mL) was added a freshly prepared solution of buffered HF·pyridine (825 μL, prepared by adding 75 μL of HF·pyridine to 150 μL of pyridine in 600 μL of THF) at 4 °C. The reaction mixture was stirred for 6 h at the same temperature, then diluted with water (5 mL) and warmed to room temperature. The mixture was extracted with EtOAc (10 mL × 2) and $CHCl_3$ (10 mL × 2). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, 20% → 50% EtOAc/hexane containing 0.1% AcOH) to afford a *seco*-acid (28.9 mg, quantitatively) as a clear oil. To a solution of the *seco*-acid (28.9 mg, 0.0358 mol) and Et_3N (149 μL, 1.074 mmol, 30 equiv) in toluene (3.8 mL) was added 2,4,6-trichlorobenzoyl chloride (102 μL, 0.716 mmol, 20 equiv) at room temperature. The mixture was stirred at room temperature for 3 h and then diluted with toluene (20 mL). The supernatant of the mixture was added dropwise to a solution of DMAP (218 mg, 1.79 mmol, 50 equiv) in toluene (42 mL) over 5 h. The anhydride flask was rinsed twice with toluene (2 mL) (each rinse was added in one portion to the reaction mixture). After an additional 1 h of stirring at room temperature, saturated aqueous $NaHCO_3$ (30 mL) was added and the resulting biphasic mixture was poured into EtOAc (30 mL). The organic layer was separated and the aqueous layer was extracted with EtOAc (30 mL × 3). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, 5% → 10% EtOAc/hexane) to afford the lactone (18.1 mg, 0.0265 mmol, 74% in two steps) as a clear oil. To 20% $Pd(OH)_2/C$ (3.3 mg) in a flask was added a solution of the lactone (5.0 mg, 7.3 μmol) in EtOAc (0.5 mL) at room temperature. The mixture was vigorously stirred under an H_2 atmosphere at room temperature for 20 min. The mixture was filtered, and the filtrate was concentrated in vacuo. The residue was purified by HPLC (column, YMC-Pack SIL SL12S05-2510WT; solvent, *i*-PrOH/

dx.doi.org/10.1021/jm300566h | J. Med. Chem. 2012, 55, 5614−5626

$CHCl_3$/hexane = 7.5:12.5:80; flow rate, 3.0 mL/min; pressure, 600 psi; UV detector, 254 nm; retention time, 16.0 min) to afford **4** (2.8 mg, 5.56 μmol, 76%) as a clear oil. ^{1}H NMR (500 MHz, 295.1 K, $CDCl_3$, 0.011 M) δ 0.81 (3H, d, *J* = 6.5 Hz, H-22), 0.85 (3H, s, H-24), 0.99 (3H, s, H-23), 1.24 (1H, t, *J* = 12.7 Hz, H-5a), 1.32 (1H, dd, *J* = 13.8, 4.4 Hz, H-5b), 1.34−1.44 (3H, m, H-10a, H-12a, H-13a), 1.44−1.56 (2H, m, H-12b, H-13b), 1.56−1.74 (4H, m, H-4, H-10b, H_2-14), 1.65 (1H, dd, *J* = 15.6, 4.2 Hz, H-8a), 2.35 (1H, dd, *J* = 12.9, 10.9 Hz, H-2a), 2.39 (1H, t, *J* = 5.9 Hz, OH), 2.44 (1H, d, *J* = 15.6 Hz, H-8b), 2.57 (2H, t, *J* = 7.6 Hz, H-15), 2.74 (1H, dd, *J* = 16.9, 3.3 Hz, H-26a), 2.78 (1H, dd, *J* = 12.9, 2.8 Hz, H-2b), 2.83 (1H, dd, *J* = 16.9, 11.5 Hz, H-26b), 3.48 (1H, td, *J* = 10.6, 2.8 Hz, H-3), 3.76 (2H, m, H-28), 4.22 (1H, m, H-11), 5.20 (2H, m, H-9, H-27), 6.38 (1H, br s, PhOH), 6.67 (1H, dd, *J* = 8.0, 2.4 Hz, H-19), 6.73 (1H, d, *J* = 7.9 Hz, H-21), 6.83 (1H, s, H-17), 7.13 (1H, t, *J* = 7.8 Hz, H-20) ppm. ^{13}C NMR (125 MHz, 296.1 K, $CDCl_3$, 0.011 M) δ 17.38 (C-22), 22.03 (C-23), 24.11 (C-13), 25.18 (C-8), 25.89 (C-24), 29.58 (C-14), 31.54 (C-4), 34.49 (C-12), 34.91 (C-10), 35.07 (C-15), 37.01 (C-26), 38.14 (C-6), 40.76 (C-2), 43.97 (C-5), 63.21 (C-11), 64.25 (C-28), 68.67 (C-9), 72.28 (C-27), 76.64 (C-3), 100.29 (C-7), 112.61 (C-19), 115.09 (C-21), 120.73 (C-17), 129.36 (C-20), 144.58 (C-16), 156.17 (C-18), 169.40 (C-25), 173.11 (C-1) ppm. HR-FAB-MS (matrix, *m*-nitrobenzyl alcohol): *m*/*z* = 505.2811 ([MH]$^+$, calcd for $C_{28}H_{41}O_8$ 505.2801). $[\alpha]^{30.0}_D$ +38.9° (*c* 0.28, $CHCl_3$).

Synthesis of 16. To a solution of KO-*t*-Bu (2.34 g, 20.90 mmol, 2.02 equiv) and *trans*-2-butene (excess) in dry THF (50 mL) was added 1.6 M *n*-BuLi in hexane (11.8 mL, 18.83 mmol, 1.82 equiv) at −78 °C under an Ar atmosphere. The resulting yellow mixture was stirred at −40 °C for 15 min. The reaction mixture was recooled to −78 °C, and a solution of (−)-*B*-methoxydiisopinocampheylborane (5.95 g, 18.83 mmol, 1.82 equiv) in dry THF (10 mL) was added. The resulting colorless reaction mixture was stirred at −78 °C for 30 min. BF_3·Et_2O (3.18 mL, 25.05 mmol, 2.42 equiv) was added rapidly followed immediately by a solution of **15** (2.79 g, 10.35 mmol) in 10 mL of dry THF. After 2 h of stirring at −78 °C, the reaction mixture was warmed to room temperature. The reaction was then quenched by addition of 1 M aqueous NaOH (36 mL) followed by 30% aqueous H_2O_2 (8 mL). The resulting mixture was stirred for 14 h at room temperature. The organic layer was separated, and the aqueous layer was extracted with EtOAc (100 mL × 3). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by column chromatography (ODS, 70% → 90% MeOH/H_2O) to afford **16** (2.95 g, 9.12 mmol, 88%) as a clear oil [92% ee by chiral HPLC analysis (column, CHIRALCEL OJ-RH; solvent, 90% MeOH/H_2O; flow rate, 0.6 mL/min; pressure, 600 psi; UV detector, 254 nm; retention time, 29.6 min)]. ^{1}H NMR (500 MHz, 295.1 K, $CDCl_3$, 0.035 M) δ 1.02 (3H, d, *J* = 6.9 Hz), 1.40 (2H, m), 1.52 (1H, d, *J* = 4.2 Hz), 1.51−1.55 (2H, m), 1.63 (2H, m), 2.19 (1H, m), 2.60 (2H, m), 3.38 (1H, br s), 5.05 (2H, s), 5.08−5.13 (2H, m), 5.71−5.78 (1H, m), 6.78−6.82 (3H, m), 7.19 (1H, t, *J* = 7.7 Hz), 7.30−7.45 (5H, m) ppm. ^{13}C NMR (125 MHz, 295.7 K, $CDCl_3$, 0.035 M) δ 16.30, 25.48, 31.39, 34.13, 36.00, 44.17, 69.96, 74.62, 111.87, 115.22, 116.28, 121.17, 127.51 (2C), 127.88, 128.55 (2C), 129.22, 137.26, 140.33, 144.39, 158.92 ppm. HR-FAB-MS (matrix, *m*-nitrobenzyl alcohol): *m*/*z* = 324.2090 ([M]$^+$, calcd for $C_{22}H_{28}O_2$ 324.2089). $[\alpha]^{13.4}_D$ +1.0° (*c* 0.58, $CHCl_3$).

Synthesis of 17. To a solution of **16** (1.13 g, 3.49 mmol) in THF (10 mL) was added 1 M sodium bis(trimethylsilyl)amide in THF (4.53 mL, 4.53 mmol, 1.3 equiv) dropwise at 4 °C. After 30 min of stirring, $(Boc)_2O$ (1.15 g, 5.28 mmol, 1.5 equiv) was added, and the reaction mixture was stirred for 15 min at room temperature. The reaction was quenched with brine (10 mL) and water (3 mL), and the mixture was extracted with EtOAc (50 mL × 3). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, 0.5% → 2% EtOAc/hexane) to afford a carbonate (1.28 g, 3.02 mmol, 87%) as a clear oil. To a solution of the carbonate (1.28 g, 3.02 mmol) in toluene (15 mL) was added IBr (2.32 g, 11.2 mmol, 1.5 equiv) in DCM (11 mL) at −78 °C. After the mixture was stirred for 2 h the same temperature, the reaction was quenched with a 1:1 mixture of 10% aqueous $Na_2S_2O_3$ and saturated aqueous $NaHCO_3$ (20 mL). The mixture was extracted with EtOAc (20 mL × 3). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, 10% → 20% → 40% EtOAc/hexane) to afford an iodocarbonate, which was dissolved in MeOH (10 mL). To this solution was added K_2CO_3 (853 mg, 6.18 mmol) at room temperature. After 45 min of stirring, the reaction mixture was poured into H_2O (40 mL) and EtOAc (40 mL). The organic layer was separated, and the aqueous layer was extracted with EtOAc (40 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, 15% EtOAc/hexane; ODS, 70% → 80% MeOH/H_2O) to afford **17** (437 mg, 1.10 mmol, 53% in two steps) as a clear oil. ^{1}H NMR (500 MHz, 295.1 K, $CDCl_3$, 0.054 M) δ 0.98 (3H, d, *J* = 7.1 Hz), 1.40 (2H, m), 1.55−1.75 (5H, m), 2.43 (1H, dd, *J* = 5.0, 2.7 Hz), 2.59 (2H, t, *J* = 7.8 Hz), 2.72 (1H, dd, *J* = 5.0, 4.1 Hz), 2.92 (1H, ddd, *J* = 7.7, 4.1, 2.7 Hz), 3.79 (3H, s), 4.81 (1H, m), 5.05 (2H, s), 6.77−6.81 (3H, m), 7.18 (1H, t, *J* = 7.8 Hz), 7.32−7.45 (5H, m) ppm. ^{13}C NMR (125 MHz, 295.9 K, $CDCl_3$, 0.054 M) δ 12.53, 25.08, 31.05, 31.56, 35.79, 40.07, 44.84, 52.97, 54.74, 69.95, 80.89, 111.97, 115.15, 121.15, 127.52 (2C), 127.89, 128.56 (2C), 129.24, 137.26, 144.13, 155.91, 158.93 ppm. HR-FAB-MS (matrix, *m*-nitrobenzyl alcohol): *m*/*z* = 399.2189 ([MH]$^+$, calcd for $C_{24}H_{31}O_5$ 399.2171). $[\alpha]^{16.0}_D$ +4.3° (*c* 1.29, $CHCl_3$).

Synthesis of 18. To a solution of **17** (1.02 g, 2.55 mmol) was added a solution of 28% NaOMe in MeOH (0.54 mL) at room temperature. After 2 h of stirring at 50 °C, saturated aqueous NH_4Cl (10 mL) was added and the resulting mixture was poured into EtOAc (20 mL). The organic layer was separated, and the aqueous layer was extracted with EtOAc (20 mL × 2). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, 15% → 30% EtOAc/hexane) to afford an alcohol (630 mg, 1.85 mmol, 73%) as a clear oil. To a suspended solution of the alcohol (12.7 mg, 37.4 μmol) and NaH (3.0 mg, 60% in mineral oil, 74.7 μmol) in THF−DMF (3 mL, 3:1) was added *p*-methoxybenzyl chloride (9.0 μL, 66.1 μmol) at room temperature. After 55 h of stirring, the mixture was quenched with 5% aqueous H_3PO_4 (5.0 mL). The solution was extracted with 1:1 mixture of EtOAc and hexane (5 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, 3% → 7% EtOAc/hexane) to afford **18** (16.7 mg, 36.3 μmol, 97%) as a clear oil. ^{1}H NMR (500 MHz, 295.1 K, $CDCl_3$, 0.070 M) δ 0.93 (3H, d, *J* = 7.1 Hz), 1.40 (1H, m), 1.46−1.69 (6H, m), 2.47 (1H, dd, *J* = 5.0, 2.7 Hz), 2.58 (2H, t, *J* = 7.7 Hz), 2.72 (1H, dd, *J* = 5.0, 4.1 Hz), 2.90 (1H, m), 3.43 (1H, m), 3.78 (3H, s), 4.41 (1H, d, *J* = 11.1 Hz), 4.44 (1H, d, *J* = 11.1 Hz), 5.04 (2H, s), 6.77−6.82 (3H, m), 6.86 (2H, m), 7.19 (1H, t, *J* = 7.8 Hz), 7.24 (2H, m), 7.30−7.44 (5H, m) ppm. ^{13}C NMR (125 MHz, 295.3 K, $CDCl_3$, 0.070 M) δ 11.55, 25.54, 30.69, 31.44, 36.00, 38.97, 45.39, 53.62, 55.28, 69.95, 71.48, 80.89, 111.87, 113.77 (2C), 115.19, 121.20, 127.52 (2C), 127.88, 128.55 (2C), 129.21, 129.34 (2C), 131.04, 137.26, 144.42, 158.92, 159.14 ppm. HR-FAB-MS (matrix, *m*-nitrobenzyl alcohol): *m*/*z* = 460.2619 ([M]$^+$, calcd for $C_{30}H_{36}O_4$ 460.2614). $[\alpha]^{17.5}_D$ +12.3° (*c* 1.03, $CHCl_3$).

Synthesis of 20. To a solution of **19** (653 mg, 1.96 mmol, 2 equiv) in dry THF (12 mL) was added 1.6 M *n*-BuLi in hexane (1.35 mL) at room temperature under an Ar atmosphere. After 45 min of stirring at room temperature, the mixture was cooled to 4 °C. A solution of **18** (450 mg, 0.978 mmol) in dry THF (15 mL) was then added, and the reaction mixture was stirred for 18 h at 4 °C. The reaction was quenched with saturated aqueous NH_4Cl (20 mL). The mixture was poured into EtOAc (30 mL) and H_2O (30 mL). After the organic layer was separated, the aqueous layer was extracted with EtOAc (40 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, 3% → 7% EtOAc/hexane) to afford **20** (743 mg, 0.934 mmol, 96%) as a clear oil.

dx.doi.org/10.1021/jm300566h | *J. Med. Chem.* 2012, 55, 5614−5626

^{1}H NMR (500 MHz, 295.0 K, $CDCl_3$, 0.019 M) δ 0.05 (6H, s), 0.89 (3H, d, J = 6.3 Hz), 0.89 (9H, s), 1.11 (6H, br s), 1.39−1.63 (10H, m), 1.87−2.01 (3H, m), 2.07 (1H, dd, J = 15.4, 9.2 Hz), 2.17 (1H, d, J = 15.4 Hz), 2.58 (2H, m), 2.81−2.97 (4H, m), 3.59 (2H, t, J = 6.4 Hz), 3.68 (1H, m), 3.78 (3H, s), 4.22 (1H, t, J = 8.2 Hz), 4.27 (1H, s), 4.36 (1H, d, J = 10.9 Hz), 4.47 (1H, d, J = 10.9 Hz), 5.04 (2H, s), 6.77−6.80 (3H, m), 6.84 (2H, m), 7.18 (1H, t, J = 7.8 Hz), 7.25 (2H, m), 7.30−7.44 (5H, m) ppm. ^{13}C NMR (125 MHz, 295.3 K, $CDCl_3$, 0.019 M) δ −5.21 (2C), 10.82, 18.35, 22.34 (2C, br), 22.85, 25.45, 26.01 (3C), 27.18, 27.47, 28.37, 29.38, 31.54, 32.80, 36.08, 41.43, 42.42, 45.75, 55.29, 63.71, 63.87, 69.95, 70.89, 71.21, 79.24, 111.80, 113.71 (2C), 115.20, 121.22, 127.54 (2C), 127.87, 128.54 (2C), 129.17, 129.28 (2C), 131.47, 137.28, 144.63, 158.91, 159.02 ppm. HR-FAB-MS (matrix, *m*-nitrobenzyl alcohol): m/z = 817.4328 ([M + Na]$^+$, calcd for $C_{46}H_{70}O_5S_2SiNa$ 817.4332). $[\alpha]^{18.0}_D$ +7.3° (c 0.94, $CHCl_3$).

Synthesis of 21. To a suspension of **20** (386 mg, 0.486 mmol) and 4 Å molecular sieves (1.0 g) in DCM (6 mL) was added DDQ (137 mg, 0.583 mmol, 1.2 equiv) at room temperature, causing a green color to appear. After 1 h of stirring at room temperature under an Ar atmosphere, the color gradually changed to brown with precipitation of a solid. The mixture was poured into saturated aqueous $NaHCO_3$ (6 mL), H_2O (20 mL), and EtOAc (20 mL). After the organic layer was separated, the aqueous layer was extracted with EtOAc (20 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/hexane) to afford an acetal (333 mg, 0.420 mmol, 87%) as a clear oil. To a solution of the acetal (322 mg, 0.407 mmol) in DCM (4.3 mL) and MeOH (4.3 mL) was added PPTS (204 mg, 0.813 mmol, 2 equiv) at room temperature. After the mixture was stirred for 1.5 h, the reaction was quenched with saturated aqueous $NaHCO_3$ (10 mL). The resulting mixture was extracted with EtOAc (20 mL × 3), and 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, 15% → 30% EtOAc/hexane) to afford an alcohol (226 mg, 0.333 mmol, 82%) as a clear oil. To a solution of the alcohol (213 mg, 0.314 mmol), NMO (55 mg, 0.471 mmol, 1.5 equiv), and 4 Å molecular sieves (157 mg) in DCM (0.63 mL) was added tetrapropylammonium perruthenate (5.5 mg, 0.097 mmol, 0.05 equiv). After 20 min of stirring at room temperature, the reaction mixture was diluted with DCM (2 mL) and absorbed in a small amount of silica gel. The resulting gel was directly loaded onto a silica gel column and eluted with 10% EtOAc/hexane. Concentration of the fractions gave an aldehyde (144 mg, 0.213 mmol, 68%) as a clear oil. Then 1 M $TiCl_4$ in DCM (31 μL, 31 μmol, 0.15 equiv) was diluted with DCM (0.5 mL) and cooled to 4 °C. To the $TiCl_4$ solution was added $Ti(O\text{-}i\text{-}Pr)_4$ (28 μL, 93 μmol, 0.45 equiv) at 0 °C. The mixture was warmed to room temperature and stirred for 1 h. Ag_2O (14 mg, 62 μmol, 0.3 equiv) was added in one portion, and stirring was continued with exclusion of direct light for 5 h. (*S*)-1,1′-Bi-2-naphthol (35 mg, 0.124 mmol, 0.6 equiv) was then added in one portion. After a further 2 h of stirring, the mixture was diluted with DCM (1.0 mL) to afford a stock solution (about 40 mM) of Ti catalyst. To the aldehyde (140 mg, 0.207 mmol) was added the supernatant (1.03 mL) of the above stock solution at −15 °C. After 15 min of stirring at the same temperature, allyl-$SnBu_3$ (128 μL, 0.414 mmol, 2 equiv) was added. The resulting reaction mixture was kept in a cold room for 63 h without stirring. The reaction was quenched with saturated aqueous $NaHCO_3$ (3 mL), and the mixture was poured into EtOAc (10 mL) and H_2O (5 mL). The organic layer was separated, and the aqueous layer was extracted with EtOAc (10 mL × 3). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, 10% → 20% EtOAc/hexane) to afford **21** (112 mg, 0.156 mmol, 75%) as a clear oil. ^{1}H NMR (500 MHz, 295.8 K, $CDCl_3$, 0.024 M) δ 0.93 (3H, d, J = 6.6 Hz), 1.17 (3H, br s), 1.19 (3H, br s), 1.35−2.06 (15H, m), 2.23 (1H, m), 2.38 (1H, dd, J = 16.2, 7.2 Hz), 2.48 (1H, d, J = 16.2 Hz), 2.60 (2H, m), 2.67−2.79 (2H, m), 2.83−2.93 (2H, m), 3.44−3.49 (2H, m), 3.78 (3H, s), 3.92 (1H, m), 5.04 (2H, s), 5.08−5.12 (2H, m), 5.44 (1H, s), 5.77 (1H, m), 6.79−6.83 (3H, m), 6.86 (2H, m), 7.19 (1H, t, J = 7.8 Hz), 7.30−7.44 (7H, m) ppm. ^{13}C NMR (125 MHz, 296.3 K, $CDCl_3$, 0.024 M) δ 13.21, 22.42 (br), 23.08 (br), 24.38, 24.90, 26.63, 27.52, 31.36, 32.10, 32.56 (br), 32.94, 36.00, 38.84, 41.52, 41.93, 44.45, 55.26, 63.83, 69.96, 71.47, 80.32, 81.96, 99.59, 111.88, 113.44 (2C), 115.19, 117.98, 121.20, 127.32 (2C), 127.52 (2C), 127.89, 128.56 (2C), 129.22, 131.65, 134.93, 137.26, 144.49, 158.93, 159.66 ppm. HR-FAB-MS (matrix, *m*-nitrobenzyl alcohol): m/z = 719.3813 ([MH]$^+$, calcd for $C_{43}H_{59}O_5S_2$ 719.3804). $[\alpha]^{19.2}_D$ −0.3° (c 0.37, $CHCl_3$).

Synthesis of 22. To a solution of **21** (118 mg, 0.164 mmol) in MeCN (1 mL) and THF (1 mL) were added H_2O (1 mL) and *p*-TsOH·H_2O (94 mg, 0.493 mmol, 3.0 equiv) at room temperature. The mixture was stirred at 50 °C for 5 h, and then the reaction was quenched with saturated aqueous $NaHCO_3$ (5 mL). The mixture was extracted with EtOAc (10 mL × 3). The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, 7% → 15% → 60% EtOAc/hexane) to afford a recovered substrate (65 mg, 0.091 mmol, 55%) and a triol (40 mg, 0.067 mmol, 41%) as a clear oil. To a solution of the triol (65.7 mg, 0.109 mmol) in MeCN (0.36 mL), DCM (0.36 mL), and H_2O (0.36 mL) was added $Hg(ClO_4)_2{\cdot}6H_2O$ (111 mg, 0.219 mmol, 2.0 equiv) at 4 °C. After 30 min of stirring at the same temperature, the reaction mixture was poured into EtOAc (5 mL) and saturated aqueous $Na_2S_2O_3$ (15 mL). The organic layer was separated, and the aqueous layer was extracted with EtOAc (20 mL × 2). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by column chromatography (3% → 8% EtOAc/hexane) to afford the desired spiroketal **22** (33.4 mg, 0.0679 mmol, 62%) as a clear oil. Since it was difficult to purify the undesired spiroketal, spectral data were not measured. ^{1}H NMR (500 MHz, 295.1 K, $CDCl_3$, 0.024 M) δ 0.89 (3H, s), 0.90 (3H, d, J = 7.0 Hz), 0.95 (3H, s), 1.34−1.70 (12H, m), 2.26−2.30 (3H, m), 2.60 (2H, m), 3.43 (1H, d, J = 11.1 Hz, OH), 3.73 (1H, m), 3.80 (2H, m), 5.05 (2H, s), 5.07−5.12 (2H, m), 5.79 (1H, m), 6.78−6.84 (3H, m), 7.19 (1H, t, J = 7.8 Hz), 7.30−7.45 (5H, m) ppm. ^{13}C NMR (125 MHz, 297.5 K, $CDCl_3$, 0.024 M) δ 14.03, 22.60, 24.42, 25.53, 26.30, 30.52, 31.33, 32.59, 33.37, 36.07, 36.77, 38.81, 40.93, 68.81, 69.95, 70.34, 72.43, 102.48, 111.76, 115.23, 117.85, 121.20, 127.50 (2C), 127.87, 128.55 (2C), 129.17, 134.78, 137.29, 144.67, 158.02 ppm. HR-FAB-MS (matrix, *m*-nitrobenzyl alcohol): m/z = 493.3296 ([MH]$^+$, calcd for $C_{32}H_{45}O_4$ 493.3318. $[\alpha]^{19.5}_D$ +63.2° (c 0.60, $CHCl_3$).

Synthesis of 23. Compound **22** (41.5 mg, 0.0843 mmol) was treated in a manner similar to that described for the synthesis of **13** to give **23** (60.0 mg, 0.0746 mmol, 89%). ^{1}H NMR (500 MHz, 295.7 K, $CDCl_3$, 0.017 M) δ 0.74 (3H, d, J = 6.9 Hz), 0.85 (3H, s), 0.95 (3H, s), 1.31−1.61 (10H, m), 1.66 (1H, dd, J = 15.0, 3.9 Hz), 1.67 (1H, m), 2.22 (1H, m), 2.30 (1H, m), 2.33 (1H, dd, J = 15.0, 3.2 Hz), 2.57−2.61 (3H, m), 2.64 (1H, dd, J = 15.8, 7.7 Hz), 3.38 (1H, m), 3.50 (1H, dd, J = 10.0, 5.2 Hz), 3.57 (1H, dd, J = 10.0, 5.3 Hz), 3.78 (2H, s), 3.98 (1H, m), 4.12 (1H, m), 4.53 (2H, s), 4.54 (1H, d, J = 11.0 Hz), 4.58 (1H, d, J = 11.0 Hz), 4.95−5.02 (3H, m), 5.04 (2H, s), 5.79 (1H, m), 6.78−6.85 (5H, m), 7.18 (1H, t, J = 7.8 Hz), 7.21−7.25 (3H, m), 7.27−7.47 (10H, m) ppm. ^{13}C NMR (125 MHz, 296.1 K, $CDCl_3$, 0.017 M) δ 13.06, 21.70, 24.27, 25.57, 26.36, 27.52, 31.21, 32.45, 34.16, 36.14, 36.88, 37.05, 38.10, 41.04, 55.28, 68.62, 69.94, 71.50, 71.97, 72.00, 72.26, 73.36, 74.82, 99.76, 111.76, 113.73 (2C), 115.20, 116.67, 121.21, 127.52 (2C), 127.62 (2C), 127.86, 128.11, 128.37 (2C), 128.54 (2C), 129.14 (1C), 129.41 (2C), 130.66, 135.07, 137.31, 138.20, 144.76, 158.90, 159.21, 171.90 ppm. HR-FAB-MS (matrix, *m*-nitrobenzyl alcohol): m/z = 804.4644 ([M]$^+$, calcd for $C_{51}H_{64}O_8$ 804.4601). $[\alpha]^{29.0}_D$ +33.1° (c 0.69, $CHCl_3$).

Synthesis of 24. Compound **23** (60.0 mg, 0.0746 mmol) was treated in a manner similar to that described for the synthesis of **14** to give **24** (35.5 mg, 0.0435 mmol, 62% in three steps). ^{1}H NMR (500 MHz, 295.0 K, $CDCl_3$, 0.037 M) δ 0.59 (6H, q, J = 7.9 Hz), 0.80 (3H, d, J = 6.9 Hz), 0.87 (3H, s), 0.92 (9H, t, J = 7.9 Hz), 0.95 (3H, s), 1.33−1.72 (11H, m), 1.70 (1H, dd, J = 15.3, 3.9 Hz), 2.23 (1H, dd, J = 15.3, 3.0 Hz), 2.51 (1H, dd, J = 15.8, 7.4 Hz), 2.53−2.61 (4H, m), 2.63

dx.doi.org/10.1021/jm300566h | J. Med. Chem. 2012, 55, 5614−5626

(1H, dd, J = 15.8, 5.0 Hz), 3.39 (1H, dd, J = 9.6, 6.7 Hz), 3.48 (1H, dd, J = 9.6, 5.1 Hz), 3.78−3.87 (2H, m), 4.35 (1H, m), 4.50 (1H, d, J = 11.9 Hz), 4.54 (1H, d, J = 11.9 Hz), 5.04 (1H, m), 5.05 (2H, s), 6.78−6.88 (3H, m), 7.19 (1H, t, J = 7.8 Hz), 7.27−7.47 (10H, m) ppm. ^{13}C NMR (125 MHz, 295.3 K, $CDCl_3$, 0.037 M) δ 4.91 (3C), 6.80 (3C), 13.08, 22.03, 24.39, 25.49, 26.40, 28.02, 31.28, 32.53, 33.19, 36.03, 36.84, 37.03, 40.42, 40.90, 67.90, 69.05, 69.75, 69.96, 70.93, 73.42, 74.27, 101.47, 111.87, 115.21, 121.24, 127.53 (2C), 127.72, 127.79 (2C), 127.86, 128.40 (2C), 128.54 (2C), 129.20, 137.31, 138.07, 144.51, 158.91, 171.67, 171.87 ppm. HR-FAB-MS (matrix, *m*-nitrobenzyl alcohol): m/z = 817.4714 ([MH]$^+$, calcd for $C_{48}H_{69}O_9Si$ 817.4711). $[\alpha]^{29.4}_D$ +48.5° (c 0.40, $CHCl_3$).

Synthesis of 5. Compound **24** (35.5 mg, 0.0435 mmol) was treated in a manner similar to that described for the synthesis of **4** to give a *seco*-acid (33.4 mg, quantitatively). To a solution of the *seco*-acid (30.5 mg, 0.0434 mol) and Et_3N (18 μL, 0.130 mmol, 3 equiv) in toluene (4 mL) was added 2,4,6-trichlorobenzoyl chloride (9.4 μL, 0.0652 mmol, 1.5 equiv) at room temperature. The mixture was stirred at room temperature for 4 h and then diluted with toluene (27 mL). The supernatant of the mixture was added dropwise to a solution of DMAP (79.5 mg, 0.652 mmol, 15 equiv) in toluene (47 mL) over 5 h. The anhydride flask was rinsed twice with toluene (2 mL) (each rinse was added in one portion to the reaction mixture). After an additional 1 h of stirring at room temperature, H_2O (30 mL) was added and the resulting biphasic mixture was poured into EtOAc (30 mL). The organic layer was separated, and the aqueous layer was extracted with EtOAc (30 mL × 3). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, 5% → 15% EtOAc/hexane) to afford a lactone (22.3 mg, 0.0326 mmol, 75% in two steps) as a clear oil. To 10% Pd/C (5 mg) in a flask was added a solution of the lactone (10.0 mg, 14.6 μmol) in EtOH (0.7 mL) at room temperature. The mixture was vigorously stirred under an H_2 atmosphere at room temperature for 1 h. The mixture was filtered, and the filtrate was concentrated in vacuo. The residue was purified by HPLC (column, YMC-Pack SIL SL12S05-2510WT; solvent, *i*-PrOH/$CHCl_3$/hexane = 6:14:80; flow rate, 3.0 mL/min; pressure, 500 psi; UV detector, 254 nm; retention time, 20.0 min) to afford **5** (5.7 mg, 11.3 μmol, 78%) as a clear oil. ^{1}H NMR (500 MHz, 294.9 K, $CDCl_3$, 0.009 M) δ 0.77 (3H, d, J = 7.0 Hz, H-24), 0.87 (3H, s, H-23), 0.96 (3H, s, H-22), 1.27−1.37 (2H, m, H-5a, H-12a), 1.37−1.48 (3H, m, H_2-4, H-13a), 1.48−1.61 (5H, m, H-5b, H-10, H-12b, H-13b, H-14a), 1.73 (1H, dd, J = 15.5, 4.1 Hz, H-8a), 1.75 (1H, m, H-14b), 2.31 (1H, br s, OH), 2.43 (1H, dd, J = 13.3, 10.8 Hz, H-2a), 2.52 (1H, dd, J = 15.5, 2.3 Hz, H-8b), 2.55 (2H, m, H-15), 2.58 (1H, dd, J = 13.3, 2.6 Hz, H-2b), 2.79 (1H, dd, J = 16.8, 4.5 Hz, H-26a), 2.84 (1H, dd, J = 16.8, 10.1 Hz, H-26b), 3.81 (2H, m, H-28), 3.92 (1H, tt, J = 11.0, 2.9 Hz, H-3), 3.98 (1H, td, J = 10.3, 2.1 Hz, H-11), 5.03 (1H, dd, J = 6.1, 3.4 Hz, H-9), 5.18 (1H, m, H-27), 6.35 (1H, br s, PhOH), 6.6 (1H, dd, J = 8.0, 2.5 Hz, H-19), 6.73 (1H, d, J = 7.6 Hz, H-21), 6.87 (1H, t, J = 1.9 Hz, H-17), 7.13 (1H, t, J = 7.8 Hz, H-20) ppm. ^{13}C NMR (125 MHz, 295.2 K, $CDCl_3$, 0.009 M) δ 13.11 (C-24), 21.35 (C-22), 24.00 (C-13), 25.97 (C-23), 26.51 (C-8), 27.27 (C-4), 29.18 (C-14), 31.58 (C-12), 34.57 (C-5), 35.29 (C-15), 36.75 (C-26), 36.98 (C-6), 37.57 (C-10), 42.84 (C-2), 64.09 (C-28), 67.22 (C-11), 70.64 (C-3), 72.43 (C-27), 72.93 (C-9), 100.01 (C-7), 112.55 (C-19), 115.00 (C-17), 120.66 (C-21), 129.31 (C-20), 144.81 (C-16), 156.26 (C-18), 169.71 (C-25), 172.87 (C-1) ppm. HR-FAB-MS (matrix, *m*-nitrobenzyl alcohol): m/z = 505.2828 ([MH]$^+$, calcd for $C_{28}H_{41}O_8$ 505.2801). $[\alpha]^{29.8}_D$ +76.2° (c 0.28, $CHCl_3$).

Measurements of Cell Growth Inhibition. A panel of 39 human cancer cell lines established by Yamori and colleagues[53] according to the NCI method with modifications was employed, and cell growth inhibitory activity was measured as reported previously.[74] 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.[75] 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 (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 of these values, each GI_{50} value, defined as $100[(T - T_0)/(C - T_0)] = 50$, was determined.

EBV-EA Induction Test. Human B-lymphoblastoid Raji cells (5×10^5/mL) were incubated at 37 °C under a 5% CO_2 atmosphere in 1 mL of RPMI 1640 medium (supplemented with 10% fetal bovine serum) with 4 mM sodium *n*-butyrate (a synergist) and 10, 100, or 1000 nM of each test compound for the induction test. Each test compound was added as 2 μL of a DMSO solution (5, 50, and 500 μM stock solution) along with 2 μL of DMSO; the final DMSO concentration was 0.4%. After 48 h of incubation, smears were made from the cell suspension, and the EBV-EA-expressing cells were stained by a conventional indirect immunofluorescence technique with an NPC patient's serum (a gift from Kobe University, Japan) and FITC-labeled anti-human IgG (DAKO, Glostrup, Denmark) as reported previously.[55,56] In each assay, at least 500 cells were counted and the proportion of the EA-positive cells was recorded. Cell viability exceeded 60% in all experiments except for that with TPA at 10^{-6} M (>50%).

Two-Stage Carcinogenesis Experiment. The back of each male 6-week-old ICR mice was shaved with surgical clippers. From a week after initiation by a single application of 390 nmol of DMBA in 0.1 mL of acetone, 1.7 nmol of TPA or DAT in 0.1 mL of acetone or 8.5 nmol of **5** in 0.1 mL of acetone was applied twice a week from week 1 to week 20. Each group consisted of 10 mice.

Inhibition of Specific Binding of [^{3}H]PDBu to PKC C1 Peptides. The binding of [^{3}H]PDBu to the PKCδ C1 peptides was evaluated by the procedure of Sharkey and Blumberg[64] with modifications as reported previously[59] using 50 mM Tris-maleate buffer (pH 7.4 at 4 °C), 10−20 nM PKCδ C1 peptides, 20 nM [^{3}H]PDBu (22.1 Ci/mmol, Perkin-Elmer Life Sciences), 50 μg/mL 1,2-dioleoyl-*sn*-glycero-3-phospho-L-serine (Sigma), 3 mg/mL bovine γ-globulin (Sigma), and various concentrations of inhibitors. Binding affinity was evaluated based on the concentration required to cause 50% inhibition of the specific binding of [^{3}H]PDBu, the IC_{50}, which was calculated with PriProbit 1.63 software.[76] The inhibition constant, K_i, was calculated by the method of Sharkey and Blumberg.[64] Although we used each PKC C1 peptide in the range of 10−20 nM, the concentration of the properly folded peptide was estimated to be about 3 nM on the basis of B_{max} values of Scatchard analyses reported previously.[59] Therefore, the concentration of free PDBu will not markedly vary over the dose−response curve.

■ ASSOCIATED CONTENT

Supporting Information

Chiral HPLC analysis of **16**; HPLC analysis of **4** and **5**; NMR spectra of **4**, **5**, **10**, and **22**; growth inhibitory activity of DAT, aplog-1, **4**, and **5** against 39 human cancer cell lines; K_d values of the specific [^{3}H]PDBu binding for whole PKC isozymes; K_i values for inhibition of the specific [^{3}H]PDBu binding for PKC C1 peptides and whole PKC isozymes by (−)-indolactam-V. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +81-75-753-6281. Fax: +81-75-753-6284. E-mail: irie@kais.kyoto-u.ac.jp.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by a Grant-in-Aid for Scientific Research on Innovative Areas "Chemical Biology of Natural

Products" (Grant No. 23102011 to K.I.) from The Ministry of Education, Culture, Sports, Science and Technology, Japan. We also thank the Screening Committee for Anticancer Drugs supported by a Grant-in-Aid for Scientific Research on Innovative Areas "Scientific Support Programs for Cancer Research" from The Ministry of Education, Culture, Sports, Science and Technology, Japan. This study was partly carried out with the JEOL JMS-700 MS spectrometer in the Joint Usage/Research Center (JURC) at the Institute for Chemical Research, Kyoto University, Japan.

■ ABBREVIATIONS USED

TPA, 12-*O*-tetradecanoylphorbol 13-acetate; ATX, aplysiatoxin; bryo-1, bryostatin 1; DAT, debromoaplysiatoxin; MG-MID, mean graph midpoint; EBV-EA, Epstein−Barr virus early antigen; PDBu, phorbol 12,13-dibutyrate

■ REFERENCES

(1) Berenblum, I. The Mechanism of Carcinogenesis. A Study of the Significance of Cocarcinogenic Action and Related Phenomena. *Cancer Res.* **1941**, *1*, 807−814.
(2) Castagna, M.; Takai, Y.; Kaibuchi, K.; Sano, K.; Kikkawa, U.; Nishizuka, Y. Direct Activation of Calcium-Activated, Phospholipid-Dependent Protein Kinase by Tumor-Promoting Phorbol Esters. *J. Biol. Chem.* **1982**, *257*, 7847−7851.
(3) Nishizuka, Y. Protein Kinase C and Lipid Signaling for Sustained Cellular Responses. *FASEB J.* **1995**, *9*, 484−496.
(4) Newton, A. C. Protein Kinase C: Structural and Spatial Regulation by Phosphorylation, Cofactors, and Macromolecular Interactions. *Chem. Rev.* **2001**, *101*, 2353−2364.
(5) Fujiki, H.; Sugimura, T. New Classes of Tumor Promoters: Teleocidin, Aplysiatoxin, and Palytoxin. *Adv. Cancer Res.* **1987**, *49*, 223−264.
(6) Fährmann, M. Targeting Protein Kinase C (PKC) in Physiology and Cancer of the Gastric Cell System. *Curr. Med. Chem* **2008**, *15*, 1175−1191.
(7) Griner, E. M.; Kazanietz, M. G. Protein Kinase C and Other Diacylglycerol Effectors in Cancer. *Nat. Rev. Cancer* **2007**, *7*, 281−294.
(8) Mackay, H. J.; Twelves, C. J. Targeting the Protein Kinase C Family: Are We There Yet? *Nat. Rev. Cancer* **2007**, *7*, 554−562.
(9) Teicher, B. A. Protein Kinase C as a Therapeutic Target. *Clin. Cancer Res.* **2006**, *12*, 5336−5345.
(10) Ishii, H.; Jirousek, M. R.; Koya, D.; Takagi, C.; Xia, P.; Clermont, A.; Burselll, S. E.; Kern, T. S.; Ballas, L. M.; Heath, W. F.; Stramm, L. E.; Feener, E. P.; King, G. L. Amelioration of Vascular Dysfunctions in Diabetic Rats by an Oral PKCβ Inhibitor. *Science* **1996**, *272*, 728−731.
(11) Savage, M. J.; Trusko, S. P.; Howland, D. S.; Pinsker, L. R.; Mistretta, S.; Reaume, A. G.; Greenberg, B. D.; Siman, R.; Scott, R. W. Turnover of Amyloid β-Protein in Mouse Brain and Acute Reduction of Its Level by Phorbol Ester. *J. Neurosci.* **1998**, *18*, 1743−1752.
(12) Mach, U. R.; Lewin, N. E.; Blumberg, P. M.; Kozikowski, A. P. Synthesis and Pharmacological Evaluation of 8- and 9-Substituted Benzolactam-V8 Derivatives as Potent Ligands for Protein Kinase C, a Therapeutic Target for Alzheimer's Disease. *ChemMedChem* **2006**, *1*, 307−314.
(13) Choi, D.-S.; Wang, D.; Yu, G.-Q.; Zhu, G.; Kharazia, V. N.; Paredes, J. P.; Chang, W. S.; Deitchman, J. K.; Mucke, L.; Messing, R. O. PKCε Increases Endothelin Converting Enzyme Activity and Reduces Amyloid Plaque Pathology in Transgenic Mice. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 8215−8220.
(14) Roebuck, K. A.; Gu, D. S.; Kagnoff, M. F. Activating Protein-1 Cooperates with Phorbol Ester Activation Signals To Increase HIV-1 Expression. *AIDS* **1996**, *10*, 819−826.
(15) Pettit, G. R.; Herald, C. L.; Doubek, D. L.; Herald, D. L.; Arnold, E.; Clardy, J. Isolation and Structure of Bryostatin 1. *J. Am. Chem. Soc.* **1982**, *104*, 6846−6848.
(16) Hale, K. J.; Hummersone, M. G.; Manaviazar, S.; Frigerio, M. The Chemistry and Biology of the Bryostatin Antitumour Macrolides. *Nat. Prod. Rep.* **2002**, *19*, 413−453.
(17) Hennings, H.; Blumberg, P. M.; Pettit, G. R.; Herald, C. L.; Shores, R.; Yuspa, S. H. Bryostatin 1, an Activator of Protein Kinase C, Inhibits Tumor Promotion by Phorbol Esters in SENCAR Mouse Skin. *Carcinogenesis* **1987**, *8*, 1343−1346.
(18) Kazanietz, M. G.; Lewin, N. E.; Gao, F.; Pettit, G. R.; Blumberg, P. M. Binding of [26-^{3}H]bryostatin 1 and Analogs to Calcium-Dependent and Calcium-Independent Protein Kinase C Isozymes. *Mol. Pharmacol.* **1994**, *46*, 374−379.
(19) Propper, D. J.; Macaulay, V.; O'Byrne, K. J.; Braybrooke, J. P.; Wilner, S. M.; Ganesan, T. S.; Talbot, D. C.; Harris, A. L. A Phase II Study of Bryostatin 1 in Metastatic Malignant Melanoma. *Br. J. Cancer* **1998**, *78*, 1337−1341.
(20) Zonder, J. A.; Shields, A. F.; Zalupski, M.; Chaplen, R.; Heilbrun, L. K.; Arlauskas, P.; Philip, P. A. A Phase II Trial of Bryostatin 1 in the Treatment of Metastatic Colorectal Cancer. *Clin. Cancer Res.* **2001**, *7*, 38−42.
(21) Ajani, J. A.; Jiang, Y.; Faust, J.; Chang, B. B.; Ho, L.; Yao, J. C.; Rousey, S.; Dakhil, S.; Cherny, R. C.; Craig, C.; Bleyer, A. A Multi-Center Phase II Study of Sequential Paclitaxel and Bryostatin-1 (NSC 339555) in Patients with Untreated, Advanced Gastric or Gastro-esophageal Junction Adenocarcinoma. *Invest. New Drugs* **2006**, *24*, 353−357.
(22) Koutcher, J. A.; Motwani, M.; Zakian, K. L.; Li, X. K.; Matei, C.; Dyke, J. P.; Ballon, D.; Yoo, H. H.; Schwartz, G. K. The in Vivo Effect of Bryostatin-1 on Paclitaxel-Induced Tumor Growth, Mitotic Entry, and Blood Flow. *Clin. Cancer Res.* **2000**, *6*, 1498−1507.
(23) Ku, G. Y.; Ilson, D. H.; Schwartz, L. H.; Capanu, M.; O'Reilly, E.; Shah, M. A.; Kelsen, D. P.; Schwartz, G. K. Phase II Trial of Sequential Paclitaxel and 1 h Infusion of Bryostatin-1 in Patients with Advanced Esophageal Cancer. *Cancer Chemother. Pharmacol.* **2008**, *62*, 875−880.
(24) Barr, P. M.; Lazarus, H. M.; Cooper, B. W.; Schluchter, M. D.; Panneerselvam, A.; Jacobberger, J. W.; Hsu, J. W.; Janakiraman, N.; Simic, A.; Dowlati, A.; Remick, S. C. Phase II Study of Bryostatin 1 and Vincristine for Aggressive Non-Hodgkin Lymphoma Relapsing after an Autologous Stem Cell Transplant. *Am. J. Hematol.* **2009**, *84*, 484−487.
(25) Schaufelberger, D. E.; Koleck, M. P.; Beutler, J. A.; Vatakis, A. M.; Alvarado, A. B.; Andrew, P.; Marzo, L. V.; Muschik, G. M.; Roach, J.; Ross, J. T.; Lebherz, W. B.; Reeves, M. P.; Eberwein, R. M.; Rodgers, L. L.; Testerman, R. P.; Snader, K. M.; Forenza, S. The Large-Scale Isolation of Bryostatin 1 from *Bugula neritina* following Current Good Manufacturing Practices. *J. Nat. Prod.* **1991**, *54*, 1265−1270.
(26) Kageyama, M.; Tamura, T.; Nantz, M. H.; Roberts, J. C.; Somfai, P.; Whritenour, D. C.; Masamune, S. Synthesis of Bryostatin 7. *J. Am. Chem. Soc.* **1990**, *112*, 7407−7408.
(27) Evans, D. A.; Carter, P. H.; Carreira, E. M.; Charette, A. B.; Prunet, J. A.; Lautens, M. Total Synthesis of Bryostatin 2. *J. Am. Chem. Soc.* **1999**, *121*, 7540−7552.
(28) Ohmori, K.; Ogawa, Y.; Obitsu, T.; Ishikawa, Y.; Nishiyama, S.; Yamamura, S. Total Synthesis of Bryostatin 3. *Angew. Chem., Int. Ed.* **2000**, *39*, 2290−2294.
(29) Halford, B. The Bryostatin's Tale. *Chem. Eng. News* **2011**, No. October 24, 11−19.
(30) Wender, P. A.; Baryza, J. L.; Bennett, C. E.; Bi, F. C.; Brenner, S. E.; Clarke, M. O.; Horan, J. C.; Kan, C.; Lacôte, E.; Lippa, B.; Nell, P. G.; Turner, T. M. The Practical Synthesis of a Novel and Highly Potent Analogue of Bryostatin. *J. Am. Chem. Soc.* **2002**, *124*, 13648−13649.
(31) Wender, P. A.; Baryza, J. L.; Brenner, S. E.; DeChristopher, B. A.; Loy, B. A.; Schrier, A. J.; Verma, V. A. Design, Synthesis, and Evaluation of Potent Bryostatin Analogs that Modulate PKC Translocation Selectivity. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 6721−6726.
(32) Keck, G. E.; Kraft, M. B.; Truong, A. P.; Li, W.; Sanchez, C. C.; Kedei, N.; Lewin, N. E.; Blumberg, P. M. Convergent Assembly of

Highly Potent Analogues of Bryostatin 1 via Pyran Annulation: Bryostatin Look-Alikes That Mimic Phorbol Ester Function. *J. Am. Chem. Soc.* **2008**, *130*, 6660−6661.
(33) Keck, G. E.; Li, W.; Kraft, M. B.; Kedei, N.; Lewin, N. E.; Blumberg, P. M. The Bryostatin 1 A-Ring Acetate Is Not the Critical Determinant for Antagonism of Phorbol Ester-Induced Biological Responses. *Org. Lett.* **2009**, *11*, 2277−2280.
(34) Trost, B. M.; Dong, G. Total Synthesis of Bryostatin 16 Using Atom-Economical and Chemoselective Approaches. *Nature* **2008**, *456*, 485−488.
(35) Trost, B. M.; Dong, G. Total Synthesis of Bryostatin 16 Using a Pd-Catalyzed Diyne Coupling as Macrocyclization Method and Synthesis of C20-*epi*-Bryostatin 7 as a Potent Anticancer Agent. *J. Am. Chem. Soc.* **2010**, *132*, 16403−16416.
(36) Wender, P. A.; Schrier, A. J. Total Synthesis of Bryostatin 9. *J. Am. Chem. Soc.* **2011**, *133*, 9228−9231.
(37) Keck, G. E.; Poudel, Y. B.; Cummins, T. J.; Rudra, A.; Covel, J. A. Total Synthesis of Bryostatin 1. *J. Am. Chem. Soc.* **2011**, *133*, 744−747.
(38) Manaviazar, S.; Frigerio, M.; Bhatia, G. S.; Hummersone, M. G.; Aliev, A. E.; Hale, K. J. Enantioselective Formal Total Synthesis of the Antitumor Macrolide Bryostatin 7. *Org. Lett.* **2006**, *8*, 4477−4480.
(39) Lu, Y.; Woo, S. K.; Krische, M. J. Total Synthesis of Bryostatin 7 via C−C Bond-Forming Hydrogenation. *J. Am. Chem. Soc.* **2011**, *133*, 13876−13879.
(40) Nakagawa, Y.; Yanagita, R. C.; Hamada, N.; Murakami, A.; Takahashi, H.; Saito, N.; Nagai, H.; Irie, K. 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. *J. Am. Chem. Soc.* **2009**, *131*, 7573−7579.
(41) Yanagita, R. C.; Kamachi, H.; Tanaka, K.; Murakami, A.; Nakagawa, Y.; Tokuda, H.; Nagai, H.; Irie, K. Role of the Phenolic Hydroxyl Group in the Biological Activities of Simplified Analogue of Aplysiatoxin with Antiproliferative Activity. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 6064−6066.
(42) Irie, K.; Yanagita, R. C.; Nakagawa, Y. Challenges to the Development of Bryostatin-Type Anticancer Drugs Based on the Activation Mechanism of Protein Kinase Cδ. *Med. Res. Rev* **2012**, *32*, 518−535.
(43) Nakagawa, Y.; Kikumori, M.; Yanagita, R. C.; Murakami, A.; Tokuda, H.; Nagai, H.; Irie, K. Synthesis and Biological Evaluation of the 12,12-Dimethyl Derivative of Aplog-1, an Anti-Proliferative Analog of Tumor-Promoting Aplysiatoxin. *Biosci., Biotechnol., Biochem.* **2011**, *75*, 1167−1173.
(44) Irie, K.; Kikumori, M.; Kamachi, H.; Tanaka, K.; Murakami, A.; Yanagita, R. C.; Tokuda, H.; Suzuki, N.; Nagai, H.; Suenaga, K.; Nakagawa, Y. Synthesis and Structure−Activity Studies of Simplified Analogues of Aplysiatoxin with Antiproliferative Activity like Bryostatin-1. *Pure Appl. Chem.* **2012**, *84*, 1341−1351.
(45) Kato, Y.; Scheuer, P. J. Aplysiatoxin and Debromoaplysiatoxin, Constituents of the Marine Mollusk *Stylocheilus longicauda* (Quoy and Gaimard, 1824). *J. Am. Chem. Soc.* **1974**, *96*, 2245−2246.
(46) Park, P. U.; Broka, C. A.; Johson, B. F.; Kishi, Y. Total Synthesis of Debromoaplysiatoxin and Aplysiatoxin. *J. Am. Chem. Soc.* **1987**, *109*, 6205−6207.
(47) Ide, M.; Nakata, M. Room-Temperature Metallation of 2-Substituted 1,3-Dithiane Derivatives and Subsequent Coupling with 2,3-Disubstituted Oxiranes. *Bull. Chem. Soc. Jpn.* **1999**, *72*, 2491−2499.
(48) Keck, G. E; Krishnamurthy, D. Catalytic Asymmetric Allylation Reactions: (*S*)-1-(Phenylmethoxy)-4-penten-2-ol. *Org. Synth.* **1998**, *75*, 12−18.
(49) Hanawa, H.; Uraguchi, D.; Konishi, S.; Hashimoto, T.; Maruoka, K. Catalytic Asymmetric Allylation of Aldehydes and Related Reactions with Bis(((*S*)-binaphthoxy)(isopropoxy)titanium) Oxide as a μ-Oxo-Type Chiral Lewis Acid. *Chem.—Eur. J.* **2003**, *9*, 4405−4413.
(50) Inanaga, J.; Hirata, K.; Saeki, H.; Katsuki, T.; Yamaguchi, M. A Rapid Esterification by Means of Mixed Anhydride and Its Application to Large-Ring Lactonization. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989−1993.
(51) Brown, H. C.; Jadhav, P. K. Asymmetric Carbon−Carbon Bond Formation via B-Allyldiisopinocampheylborane. Simple Synthesis of Secondary Homoallylic Alcohols with Excellent Enantiomeric Purities. *J. Am. Chem. Soc.* **1983**, *105*, 2092−2093.
(52) Duan, J. J.-W.; Smith, A. B., III. Iodine Monobromide (IBr) at Low Temperature: Enhanced Diastereoselectivity in Electrophilic Cyclizations of Homoallylic Carbonates. *J. Org. Chem.* **1993**, *58*, 3703−3711.
(53) Yamori, T.; Matsunaga, A.; Sato, S.; Yamazaki, K.; Komi, A.; Ishizu, K.; Mita, I.; Edatsugi, H.; Matsuba, Y.; Takezawa, K.; Nakanishi, O.; Kohno, H.; Nakajima, Y.; Komatsu, H.; Andoh, T.; Tsuruo, T. 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 Res.* **1999**, *59*, 4042−4049.
(54) Partition Coefficient (*n*-Octanol/Water), High Performance Liquid Chromatography (HPLC) Method. In *OECD Guidelines for Testing of Chemicals. Section 1: Physical-Chemical Properties*; OECD Publishing: Paris, 1989; Test No. 117.
(55) Ito, Y.; Yanase, S.; Fujita, J.; Harayama, T.; Takashima, M.; Imanaka, H. A Short-Term in Vitro Assay for Promoter Substances Using Human Lymphoblastoid Cells Latently Infected with Epstein−Barr Virus. *Cancer Lett.* **1981**, *13*, 29−37.
(56) zur Hausen, H.; Bornkamm, G. W.; Schmidt, R.; Hecker, E. Tumor Initiators and Promoters in the Induction of Epstein−Barr Virus. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 782−785.
(57) Suganuma, M.; Fujiki, H.; Tahira, T.; Cheuk, C.; Moore, R. E.; Sugimura, T. Estimation of Tumor Promoting Activity and Structure−Function Relationships of Aplysiatoxins. *Carcinogenesis* **1984**, *5*, 315−318.
(58) Nakamura, H.; Kishi, Y.; Pajares, M. A.; Rando, R. R. Structural Basis of Protein Kinase C Activation by Tumor Promoters. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 9672−9676.
(59) Shindo, M.; Irie, K.; Nakahara, A.; Ohigashi, H.; Konishi, H.; Kikkawa, U.; Fukuda, H.; Wender, P. A. 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. *Bioorg. Med. Chem.* **2001**, *9*, 2073−2081.
(60) Quest, A. F. G.; Bell, R. M. 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, and Divalent Cations. *J. Biol. Chem.* **1994**, *269*, 20000−20012.
(61) Raghunath, A.; Ling, M.; Larsson, C. The Catalytic Domain Limits the Translocation of Protein Kinase Cα in Response to Increases in Ca^{2+} and Diacylglycerol. *Biochem. J.* **2003**, *370*, 901−912.
(62) Szállási, Z.; Bögi, K.; Gohari, S.; Biro, T.; Ács, P.; Blumberg, P. M. Non-Equivalent 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. *J. Biol. Chem.* **1996**, *271*, 18299−18301.
(63) Masuda, A.; Irie, K.; Nakagawa, Y.; Ohigashi, H. Binding Selectivity of Conformationally Restricted Analogues of (−)-Indolactam-V to the C1 Domains of Protein Kinase C Isozymes. *Biosci., Biotechnol., Biochem* **2002**, *66*, 1615−1617.
(64) Sharkey, N. A.; Blumberg, P. M. Highly Lipophilic Phorbol Esters as Inhibitors of Specific [^{3}H]Phorbol 12,13-Dibutyrate Binding. *Cancer Res.* **1985**, *45*, 19−24.
(65) Irie, K.; Nakagawa, Y.; Ohigashi, H. Indolactam and Benzolactam Compounds as New Medicinal Leads with Binding Selectivity for C1 Domains of Protein Kinase C Isozymes. *Curr. Pharm. Des* **2004**, *10*, 1371−1385.
(66) Haughian, J. M.; Reno, E. M.; Thorne, A. M.; Bradford, A. P. Protein Kinase Cα-Dependent Signaling Mediates Endometrial Cancer Cell Growth and Tumorigenesis. *Int. J. Cancer* **2009**, *125*, 2556−2564.

(67) Mandil, R.; Ashkenazi, E.; Blass, M.; Kronfeld, I.; Kazimirsky, G.; Rosenthal, G.; Umansky, F.; Lorenzo, P. S.; Blumberg, P. M.; Brodie, C. Protein Kinase Cα and Protein Kinase Cδ Play Opposite Roles in the Proliferation and Apoptosis of Glioma Cells. *Cancer Res.* **2001**, *61*, 4612−4619.
(68) Lu, Z.; Hornia, A.; Jiang, Y.-W.; Zang, Q.; Ohno, S.; Foster, D. A. Tumor Promotion by Depleting Cells of Protein Kinase Cδ. *Mol. Cell. Biol.* **1997**, *17*, 3418−3428.
(69) Reddig, P. J.; Dreckschmidt, N. E.; Ahrens, H.; Simsiman, R.; Tseng, C.-P.; Zou, J.; Oberley, T. D.; Verma, A. K. Transgenic Mice Overexpressing Protein Kinase Cδ in the Epidermis Are Resistant to Skin Tumor Promotion by 12-*O*-Tetradecanoylphorbol-13-acetate. *Cancer Res.* **1999**, *59*, 5710−5718.
(70) Jeffrey, A. M.; Liskamp, R. M. J. Computer-Assisted Molecular Modeling of Tumor Promoters: Rationale for the Activity of Phorbol Esters, Teleocidin B, and Aplysiatoxin. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 241−245.
(71) Zhang, G.; Kazanietz, M. G.; Blumberg, P. M.; Hurley, J. H. Crystal Structure of the Cys2 Activator-Binding Domain of Protein Kinase Cδ in Complex with Phorbol Ester. *Cell* **1995**, *81*, 917−924.
(72) Bertolini, T. M.; Giorgione, J.; Harvey, D. F.; Newton, A. C. Protein Kinase C Translocation by Modified Phorbol Esters with Functionalized Lipophilic Regions. *J. Org. Chem.* **2003**, *68*, 5028−5036.
(73) Irie, K.; Oie, K.; Nakahara, A.; Yanai, Y.; Ohigashi, H.; Wender, P. A.; Fukuda, H.; Konishi, H.; Kikkawa, U. 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. *J. Am. Chem. Soc.* **1998**, *120*, 9159−9167.
(74) Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolff, A.; Gray-Goodrich, M.; Campbell, H.; Mayo, J.; Boyd, M. Feasibility of a High-Flux Anticancer Drug Screen Using a Diverse Panel of Cultured Human Tumor Cell Lines. *J. Natl. Cancer Inst.* **1991**, *83*, 757−766.
(75) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. New Colorimetric Cytotoxicity Assay for Anticancer-Drug Screening. *J. Natl. Cancer Inst.* **1990**, *82*, 1107−1112.
(76) Sakuma, M. Probit Analysis of Preference Data. *Appl. Entomol. Zool.* **1998**, *33*, 339−347.