Articles

Design and Synthesis of 8-Octyl-benzolactam-V9, a Selective Activator for Protein Kinase Cϵ and η

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Conventional (α, βI, βII, γ) and novel (δ, ϵ, η, θ) protein kinase C (PKC) isozymes are main targets of tumor promoters, such as phorbol esters and indolactam-V (ILV). We have recently found that 1-hexyl derivatives of indolinelactam-V (**2**, **3**), in which the indole ring of ILV was replaced with the indoline ring, showed a binding preference for novel PKCs over conventional PKCs. To develop a new ILV analogue displaying increased synthetic accessibility and improved binding selectivity for novel PKCs, we have designed 8-octyl-benzolactam-V9 (**4**), a simple analogue without the pyrrolidine moiety of **2** and **3**. Compound **4** showed significant binding selectivity for isolated C1B domains of novel PKCs. Moreover, **4** translocated PKCϵ and η from the cytoplasm to the plasma membrane of HeLa cells at 1 μM, whereas other PKC isozymes did not respond even at 10 μM. These results indicate that **4** could be a selective activator for PKCϵ and η.

Introduction

Protein kinase C (PKC) is a family of serine/threonine kinases involved in many cellular processes, such as cell cycle regulation, gene expression, cell differentiation, and apoptosis.[1,2] PKC is also recognized as a main target of tumor promoters,[3,4] such as phorbol esters and indolactam-V (ILV).[5,6] Eight PKC isozymes have been identified to bind tumor promoters (Figure 1): calcium-dependent conventional PKCs α, βI, βII, and γ and calcium-independent novel PKCs δ, ϵ, η, and θ.[7] Each contains two binding sites of tumor promoters, designated as C1A and C1B domains, with a cysteine-rich sequence of 50 amino acid residues.[8] Although the mechanism of tumor promotion is still under investigation, recent studies have revealed that several novel PKCs (δ, ϵ, η) might be involved in tumor promotion[9−11] and that they are activated by tumor promoters that bind to the C1B domains.[12−14] Selective activators for novel PKCs with binding preferences for their C1B domains would facilitate the analysis of the mechanism of tumor promotion.

We have recently synthesized isolated C1 domains of all PKC isozymes (C1 peptides) in over 98% purity[15,16] and found that the 1-hexyl derivative of ILV (**1**, Figure 2) showed moderate selectivity for the C1B domains of novel PKCs using the C1 peptide library.[17,18] The binding selectivity for novel PKCs could be improved by replacing the indole ring of **1** with an indoline ring, as exemplified by 1-hexyl-indolinelactam-V (**2**, **3**).[17,18] However, their unique 1,3,4-trisubstituted indoline structures hinder their synthetic accessibility and utility as lead compounds for novel PKC-specific activators.

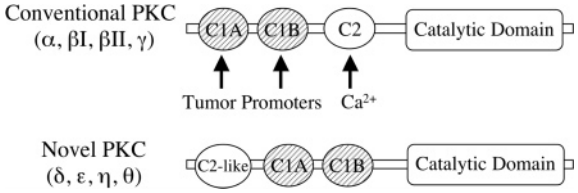

Figure 1. PKC isozymes.

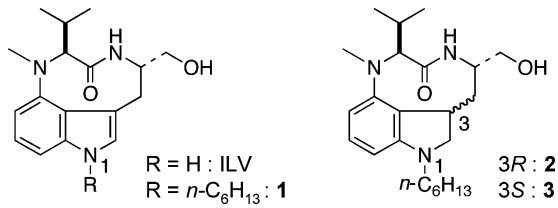

Figure 2. Indolactam-V (ILV) and its derivatives (**1**−**3**).

In this article, we describe the design and synthesis of a simplified analogue of **2** and **3**, 8-octyl-benzolactam-V9 (**4**) without a pyrrolidine moiety. Compound **4** could be easily synthesized from commercially available 2-bromo-6-nitrotoluene and acted selectively upon PKCϵ and η by binding to their C1B domains. These results suggest that **4** might be a useful tool to analyze the roles of these isozymes on tumor promotion.

Results and Discussion

Both (3*R*)- and (3*S*)-1-hexyl-indolinelactam-V compounds (**2**, **3**) showed significant binding selectivity for C1B peptides of novel PKCs despite a large difference in their main conformations.[17,18] The conformation of the 3*R* isomer (**2**) was characterized by a *cis*-amide geometry, whereas the 3*S* isomer (**3**) existed in a *trans*-amide conformation in $CDCl_3$ (Figure 3).[17] However, quite a small amount of the *cis*-amide conformer of **3** was detected in CD_3OD (data not shown). Because the ILV

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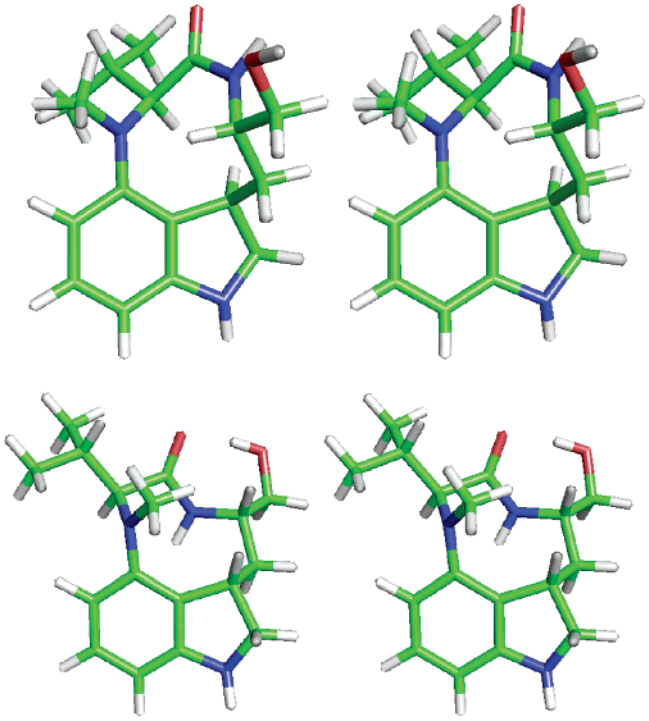


Figure 3. Conformations of indolinelactam-V compounds. Top panel: 3*R* isomer (**2**). Bottom panel: 3*S* isomer (**3**).

analogues could bind to PKC C1 peptides as a *cis*-amide conformer,[18−20] these results suggest that **3** would bind to PKC isozymes in a *cis*-amide conformation as well as **2**, and that the deletion of π electrons in **1** at positions 2 and 3 might be responsible for the selective binding of **2** and **3** to the C1B domains of novel PKCs. Because the major conformation of **3** is the *trans*-amide, **3** might bind to the C1B peptides of novel PKC isozymes as the *trans*-amide conformation. However, this is unlikely because the *trans*-amide restricted analogue of indolactam-V (5-chloro-1-hexyl-indolactam-V10) that we recently synthesized was completely inactive,[18] suggesting that **3** could bind to the C1B peptides as the *cis*-amide conformation. On the basis of this consideration, we designed 8-octyl-benzolactam-V9 (**4**) (Figure 4). In **4**, the replacement of the indole ring with a benzene ring could reduce the structural complexity and, at the same time, remove the π electrons at positions 2 and 3. The octyl group at position 8 of **4** would compensate for the decreased hydrophobicity derived from the removal of the pyrrole moiety of **1**. Calculated log *P* values of 1-hexyl-ILV (**1**) and **4** were 5.83 and 7.20, respectively, suggesting that **4** is more hydrophobic than **1**.

8-Octyl-benzolactam-V9 (**4**) was synthesized from 2-bromo-6-nitrotoluene (**5**) as shown in Scheme 1. After bromination at the benzyl position of **5**, a substitution reaction with sodium diethyl malonate gave **6** (89% in two steps). The diester was then hydrolyzed and decarboxylated under acidic conditions. The resulting mono-carboxylic acid was converted to the ethyl ester. A reduction of the ester group followed by the protection of the resulting hydroxyl group with an acetyl group afforded **7** (99% in four steps). A modified Sonogashira reaction[21] of **7** with 1-octyne gave the coupling product (56%), the triple bond, and the nitro group, which were reduced by hydrogenation. The resulting aniline derivative was formylated to give **9** (99% in two steps). After the reduction of the formyl group and the deprotection of the acetyl group of **9** (99% in two steps), the valine unit was stereoselectively introduced by the S_N2 reaction of **10** with Kogan's triflate[22] (73%). Dess−Martin oxidation of

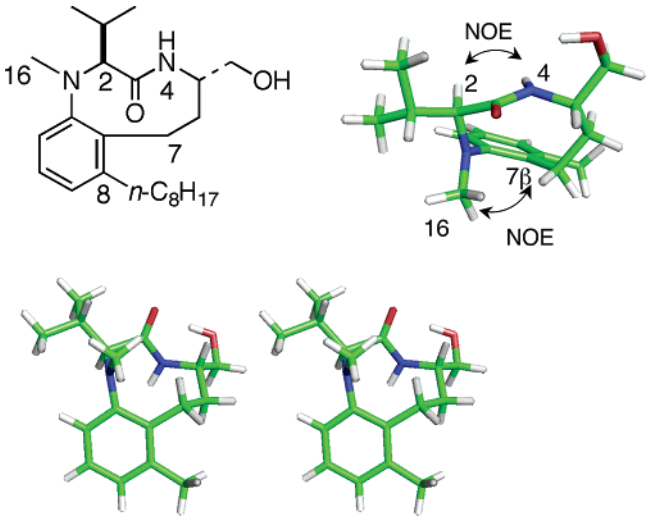
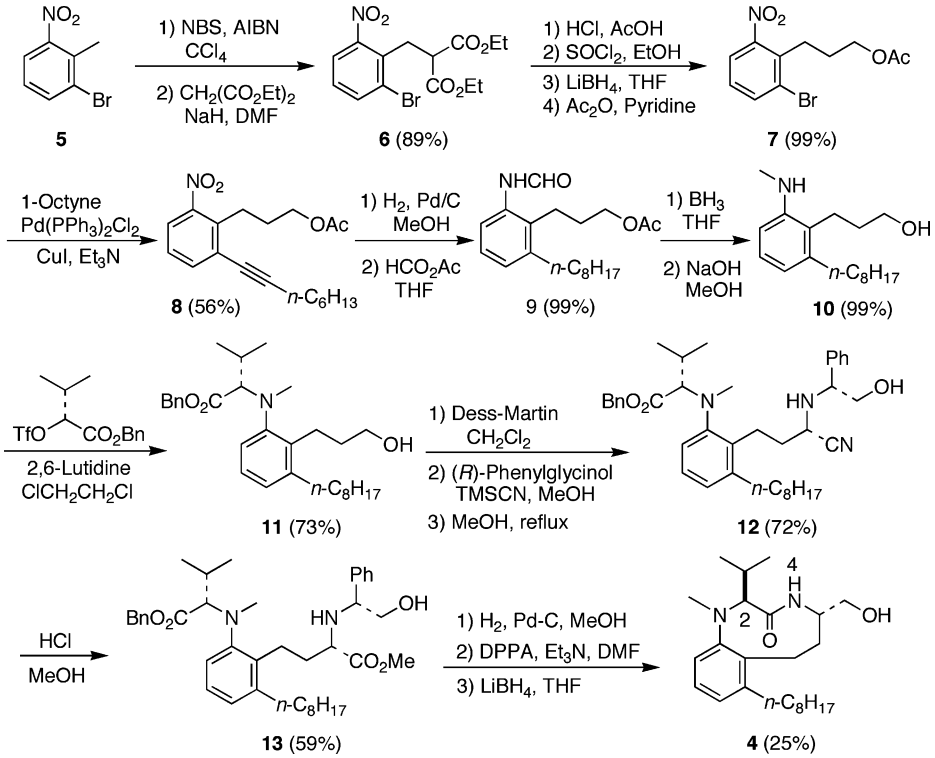


Figure 4. Major conformer of 8-octyl-benzolactam-V9 (**4**) and its structure. The conformation of **4** was determined by MM2 and PM3 calculations on the basis of the indicated NOE interactions followed by the optimization using a Hartree−Fock calculation with 6-31G*. The octyl group was displayed as a methyl group for convenience.

11 gave the aldehyde, which was subjected to an asymmetric Strecker reaction[23] to stereoselectively afford the (*S*)-amino nitrile (**12**) (72%). The cyano group of **12** was then converted to the methyl ester with HCl-saturated MeOH (59%). The removal of the chiral auxiliary group and the deprotection of the benzyl group was accomplished by hydrogenation. The formation of the lactam ring using DPPA followed by the reduction of the methyl ester gave **4** (25% in three steps).

The ^{1}H NMR spectrum showed that **4** existed as two conformers at room temperature in $CDCl_3$ in the ratio 1:3.7. The conformer ratio of **4** is solvent- and concentration-dependent. For example, the ratio of the major to minor conformers was 4.3:1 in $CDCl_3$ (0.031 M), whereas it was 3.4:1 in CD_3OD (0.031 M) (Supporting Information). A set of nonexchangeable protons almost coalesced at 55 °C in $CDCl_3$ and at 70 °C in pyridine-d_5 (Supporting Information). The signal assignments in **4** were carried out in $CDCl_3$ using ^{1}H-^{1}H COSY and scalar heteronuclear experiments (HMBC and HMQC). The highfield shifts of H-2 (δ 2.85) and H-4 (δ 4.58) signals of the major conformer compared with those of the minor conformer (δ 3.50 and 5.65, respectively) and a significant NOE enhancement between them (δ 2.85 and 4.58) (Supporting Information), which were characteristic of the *trans*-amide conformation in **3**,[17] were observed in the major conformer of **4**, indicating that the major conformation of **4** was similar to that of **3** with a *trans*-amide geometry (Figure 4). Moreover, a distinctive NOE enhancement between H-7β (δ 2.34) and H-16 (δ 2.71) was detected. On the basis of these NOE data, we analyzed the major conformation of **4** using molecular mechanics and quantum mechanics calculations. The initial structure was calculated by MM2 with the condition that the distances between H-2 and H-4 and between H-7β and H-16 were fixed at 2 Å. The resultant structure was optimized by PM3. Further optimization was carried out by a Hartree−Fock calculation with a 6-31G* basis set. The lactam conformation of the obtained structure of **4** was almost the same as that of **3** (Figures 3 and 4). The minor conformer of **4** could not be fully assigned because of some signal overlapping and a few characteristic NOE enhancements. Because some signals of the minor conformer were a slightly broadened, a variable-temperature NMR study of **4** at 300, 273, 253, and 243 K was conducted (Supporting Information).

Scheme 1. Synthesis of 8-Octyl-benzolactam-V9 (**4**)



Although the chemical shifts of the signals for NH and OH changed, the spectrum of **4** did not qualitatively change, suggesting that the two sets of the signals could be assigned to each conformer.

To estimate the minor conformer of **4**, a conformation search was carried out using the random search method implemented in Sybyl 7.1 (Tripos, Inc.). The dielectric constant was set to 5.0, corresponding to the NMR solvent ($CHCl_3$). The octyl group of **4** was replaced with the ethyl group to simplify the calculation. As a result, we obtained 11 conformers on the basis of Itai's nomenclature (Supporting Information).[24] The global minimum conformer (sofa) was identical with the major conformer of **4** (Figure 4). In the minor conformer of **4**, only one significant NOESY cross-peak was observed between H-2 (δ 3.70) and H-11 (δ 7.13) (Supporting Information). Although both the fold and *trans*-fold-like conformers satisfied this constraint, the NOESY cross-peaks between H-4 and H-5, H-7β, or H-16 were not observed in the minor conformer of **4**, suggesting that the minor conformer might be the fold form (Supporting Information).

The binding affinities of **4** for the PKC C1 peptides were evaluated by the inhibition of the specific binding of [^{3}H]phorbol 12,13-dibutyrate (PDBu) to these peptides by the method reported previously.[14,15,25] Table 1 shows the inhibition constants (K_i) of **4** as well as **3** for the PKC C1 peptides. The binding affinities of **4** for the C1B peptides of novel PKCs were about 10-fold lower than those of **3**, probably because of the high flexibility of its lactam ring. However, the binding selectivity for these C1 peptides was quite high; **4** showed little or no binding to both the C1 peptides of conventional PKCs and the C1A peptides of novel PKCs. Because **4** as well as **3** seems to bind to the C1B domains as the *cis*-amide conformation,[18] these results indicate that the deletion of the π electrons at positions 2 and 3 of ILV analogues could be effective for increasing the binding selectivity for the C1B peptides of novel PKCs.

PKC activation is tightly coupled with its translocation from the cytoplasm to the plasma membrane.[26] The binding of a PKC activator, such as a tumor promoter, to an inactive PKC in the cytoplasm increases its membrane affinity. The membrane association causes a conformational change in PKC and results in the release of the autoinhibitory sequence from its kinase domain to activate the enzyme. Recently, Wada et al.[27] reported that a phorbol ester analogue with a glycol moiety in the ester chain at position 12 strongly bound to PKCδ but did not translocate the enzyme, indicating that the binding and activation abilities of PKC ligands do not fully correspond to each other. It is thus important to examine the translocation-inducing abilities as well as the binding abilities of PKC ligands.

The translocation assay using a fusion protein of a PKC isozyme and a green fluorescent protein (GFP) in living cells is one of the promising methods to evaluate the translocation-

Table 1. K_i Values for the Inhibition of the Specific Binding of [^{3}H]PDBu by (3*S*)-1-Hexyl-indolinelactam-V (**3**) and 8-Octyl-benzolactam-V9 (**4**)

	K_i (nM)		K_d (nM)
PKC C1 peptide	(3*S*)-1-hexyl-indolinelactam-V (**3**)[a]	8-octyl-benzolactam-V9 (**4**)	PDBu
α-C1A (72-mer)[b]	550 (44)[c]	>10000	1.1
α-C1B	>10000	>10000	5.3
β-C1A (72-mer)	1200 (27)	>10000	1.3
β-C1B	250	>10000	1.3
γ-C1A	1800 (290)	>10000	1.5
γ-C1B	1600	5200 (810)	1.2
δ-C1A	>10000	>10000	52
δ-C1B	16 (1.5)	216 (9.0)	0.53
ϵ-C1A	>10000	>10000	5.6
ϵ-C1B	14 (1.0)	510 (39)	0.81
η-C1A	>10000	>10000	4.3
η-C1B	12 (2.0)	144 (13)	0.45
θ-C1A	NT[d]	NT	>200
θ-C1B	12 (1.3)	290 (15)	0.72

[a] These data are cited from ref 17. [b] Ten residues from both N- and C-termini of the previous α-C1A and β-C1B were elongated because the solubility of the orignal 52-mer peptides was extremely low.[14] [c] Standard deviation of at least two separate experiments. [d] Not tested.

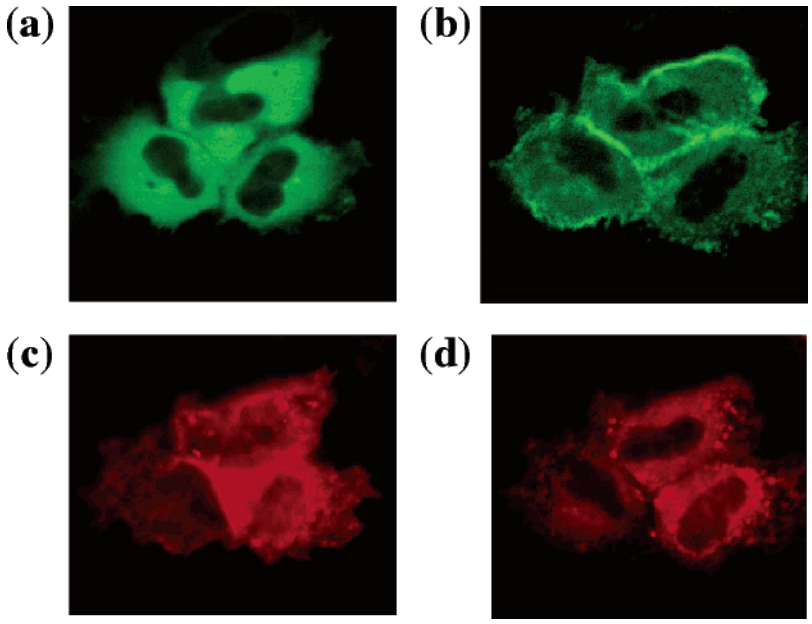


Figure 5. Translocation of GFP-tagged PKCϵ by 8-octyl-benzolactam-V9 (**4**). The fluorescence of GFP-tagged PKCϵ in HeLa cells: (a) before and (b) after stimulation by **4** (10 μM). The fluorescence of tandem DsRed2-tagged MARCKS in HeLa cells: (c) before and (d) after stimulation by **4** (10 μM).

Table 2. Translocation of GFP-Tagged PKC Isozymes Induced by Various Concentrations of (3*S*)-1-Hexyl-indolinelactam-V (**3**) Expressed by the *R* Values[a]

PKC isozymes	10 μM	1 μM	0.5 μM
PKCα	1.79 (0.33)[b]	0.97 (0.01)	NT[c]
PKCβI	2.38 (0.31)	2.39 (0.29)	0.95 (0.03)
PKCγ	1.53 (0.29)	0.96 (0.05)	NT
PKCδ	2.41 (0.41)	0.90 (0.06)	NT
PKCϵ	4.29 (1.25)	2.73 (0.18)	2.42 (0.36)
PKCη	1.92 (0.23)	1.84 (0.06)	1.01 (0.26)

[a] The values ($R = I_{pm}/I_{cyt}$) represent the ratio of the fluorescent intensities between the cytosol (I_{cyt}) and the plasma membrane (I_{pm}). The details are described in the Experimental Section. [b] Standard deviation of at least three measurements. [c] Not tested.

inducing ability of PKC ligands.[28−30] We expressed a GFP-fusion protein of each PKC isozyme in HeLa cells and examined its translocation by the stimulation of **3** and **4** at various concentrations. Membrane translocation of the GFP-fusion protein of PKCϵ induced by 10 μM **4** is shown in Figure 5a and b as a typical example. To quantify the translocation, the relative fluorescence intensity in the plasma membrane (*R*) was defined as $R = I_{pm}/I_{cyt}$, where I_{pm} and I_{cyt} are the plasma membrane fluorescence intensity and the average cytosolic fluorescence intensity, respectively. After each compound (**3**, **4**, and ILV) was added at various concentrations, the *R* values were plotted against time (Supporting Information). The maximum *R* values of **3** and **4** at various concentrations are summarized in Tables 2 and 3. Unexpectedly, significant selectivity for novel PKCs was not observed in **3**; Compound **3** (1 μM) translocated two isozymes of novel PKCs (PKCϵ, η) as well as one of conventional PKCs (PKCβI). However, compound **4** selectively translocated PKCϵ and η at 1 μM. The minimum concentration of **4** to induce PKCδ translocation was 20 μM, and conventional PKCs (PKCα, βI, γ) did not respond at the same concentration (Table 3).

The activation of PKCϵ and η was confirmed by monitoring the translocation of the myristoylated alanine-rich C-kinase substrate (MARCKS), one of the most popular PKC substrates that changes its distribution from the plasma membrane to the cytoplasm in a PKC phosphorylation-dependent manner.[31] We examined the translocation of tandem DsRed2-tagged MARCKS using HeLa cells expressing GFP-tagged PKCϵ stimulated by **4** at 1 μM. PKCϵ in the cytoplasm translocated to the plasma membrane after the stimulation of **4**, and the membrane-distributed MARCKS translocated to the cytoplasm as the membrane target of PKCϵ (Figure 5). PKCη also translocated to the plasma membrane by **4** at 1 μM and changed the distribution of MARCKS (data not shown). These results suggest that only **4** can selectively activate PKCϵ and η in living cells.

Conclusion

On the basis of the hypothesis that the deletion of π electrons at positions 2 and 3 of 1-hexyl-indolactam-V (**1**) could increase the binding selectivity for the C1B domains of novel PKCs as exemplified for 1-hexyl-indolinelactam-V compounds (**2**, **3**),[17] we designed 8-octyl-benzolactam-V9 (**4**) without the pyrrole moiety of **1**. Compound **4** showed significant binding selectivity for the C1B peptides of novel PKCs comparable to those of **2** and **3**. We have recently found that the indole ring of ILV could

Table 3. Translocation of GFP-Tagged PKC Isozymes Induced by Various Concentrations of 8-Octyl-benzolactam-V9 (**4**) Expressed by the *R* Values[a]

PKC isozymes	30 μM	20 μM	10 μM	5 μM	1 μM	0.5 μM
PKCα	0.98 (0.01)[b]	0.97 (0.01)	0.98 (0.01)	NT[c]	NT	NT
PKCβI	2.40 (0.52)	0.97 (0.01)	0.97 (0.01)	NT	NT	NT
PKCγ	0.97 (0.01)	0.97 (0.01)	0.97 (0.01)	NT	NT	NT
PKCδ	NT	2.22 (0.16)	0.95 (0.03)	NT	NT	NT
PKCϵ	NT	NT	1.96 (0.36)	2.67 (0.59)	1.66 (0.20)	1.65 (0.11)
PKCη	NT	NT	1.72 (0.11)	1.78 (0.30)	1.46 (0.12)	0.97 (0.02)

[a] The values ($R = I_{pm}/I_{cyt}$) represent the ratio of the fluorescent intensities between the cytosol (I_{cyt}) and the plasma membrane (I_{pm}). The details are described in the Experimental Section. [b] Standard deviation of at least three measurements. [c] Not tested.

be involved in the CH/π interaction with the hydrogen atom at position 4 of Pro-11 of the PKCδ C1B domain.[32] Although Pro-11 is preserved in all C1 domains of conventional and novel PKCs, the spatial position of Pro-11 in PKC isozymes might be different from each other. The selective binding of **2**, **3**, and **4** for the C1B domains of novel PKCs, thus, might reflect, in part, the difference of the CH/π interaction with Pro-11 between conventional and novel PKCs in addition to the steric hindrance at position 1.[18]

The PKC translocation assay using GFP-tagged PKC isozymes revealed that **4** could selectively activate PKCϵ and η, whereas **3** did not show any selective activation for novel PKCs. Garcia-Bermejo et al. reported that new diacylglycerol-lactones did not show any binding selectivity between PKCα and δ but selectively activated PKCα in LNCaP prostate cancer cells.[33] These results indicate that it is important to evaluate the translocation-inducing ability of PKC ligands as well as their PKC-binding ability. Compound **4** is the first PKC ligand that could selectively activate PKCϵ and η by binding to their C1B domains. Because PKCϵ and η play important roles in tumor promotion,[10,11] **4** would be useful for analyzing the mechanism of tumor promotion.

Experimental Section

General Remarks. The following spectroscopic and analytical instruments were used: UV, Shimadzu UV-2200A; $[\alpha]_D$, Jasco DIP-1000; ^{1}H NMR, Bruker ARX500, AVANCE400 (reference to TMS); HPLC, Waters Model 600E with Model 2487 UV detector; (HR) EIMS and HRMS−FAB JEOL JMS-600H. The NOESY spectrum was measured using the pulse sequence noesytp in BRUKER ARX500 with a mixing time of 800 ms (Supporting Information). Essentially similar spectra were obtained by varying the D8 parameter (500 and 300 ms) (Supporting Information). HPLC was carried out on a YMC-packed SH-342−5 (ODS, 20 mm i.d. × 150 mm) column (Yamamura Chemical Laboratory). Wakogel C-200 (silica gel, Wako Pure Chemical Industries) and YMC A60−350/250 gel (ODS, Yamamura Chemical Laboratory) were used for column chromatography. [^{3}H]PDBu (19.0 Ci/mmol) was purchased from Perkin-Elmer Life Science. All the other chemicals and reagents were purchased from chemical companies and used without further purification.

Synthesis of 8-Octyl-benzolactam-V9 (4). To a suspension of 2-bromo-6-nitrotoluene (5.0 g, 23.1 mmol) and NBS (4.5 g, 25.4 mmol) in CCl_4 (25 mL) was added AIBN (760 mg, 4.62 mmol). The reaction mixture was refluxed at 100 °C for 15 h and then filtered. The filtrate was concentrated under reduced pressure and purified by column chromatography on Wakogel C-200 using hexane and increasing amounts of EtOAc to give a benzyl bromine derivative (6.33 g, 21.1 mmol). NaH in oil (1.11 g, 27.7 mmol) was washed with hexane and suspended in dry DMF (10 mL) under an Ar atmosphere. Diethyl malonate (3.90 mL, 25.7 mmol) was added in three portions at 0 °C, and the resulting suspension was stirred at 0 °C for 10 min. A solution of the benzyl bromine derivative (6.33 g, 21.1 mmol) in DMF (20 mL) was added dropwise. The reaction mixture was stirred for 2 h at 0 °C and then poured into EtOAc and H_2O. The EtOAc layer was collected, and the aqueous layer was extracted with EtOAc. The combined EtOAc layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on Wakogel C-200 using hexane and increasing amounts of EtOAc to give **6** (7.08 g, 18.9 mmol, 89% in two steps). Compound **6**: ^{1}H NMR δ (500 MHz, 300 K, $CDCl_3$, 0.027 M): 1.23 (6H, t, J = 7.1 Hz, $-CO_2CH_2CH_3$ × 2), 3.69 (2H, d, J = 7.6 Hz, ArCH_2-), 3.82 (1H, t, J = 7.6 Hz, $-CH_2CH(CO_2Et)_2$), 4.18 (4H, dd, J = 14.2, 7.1 Hz, $-CO_2CH_2CH_3$ × 2), 7.28 (1H, t, J = 8.1 Hz, *Ar*), 7.78 (1H, d, J = 8.1 Hz, *Ar*), 7.80 (1H, d, J = 8.1 Hz, *Ar*); HRMS−FAB *m*/*z*: 374.0260 (MH^+, calcd for $C_{14}H_{17}NO_6Br$, 374.0239).

To a solution of **6** (7.01 g, 18.9 mmol) in AcOH (20 mL) was added concentrated HCl (20 mL), and the mixture was refluxed at 120 °C for 15 h. The reaction mixture was poured into H_2O and extracted with EtOAc. The collected EtOAc layer was washed with brine, dried over Na_2SO_4, and concentrated to give the crude monocarboxylic acid. The crude monocarboxylic acid in EtOH (10 mL) was added to a solution of $SOCl_2$ (10 mL) in dry ethanol (20 mL) at 0 °C. The mixture was refluxed for 1.5 h at 100 °C and then concentrated. The residue was poured into saturated aqueous $NaHCO_3$ and extracted with EtOAc. The collected EtOAc layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on Wakogel C-200 using hexane and increasing amounts of EtOAc to give the ethyl ester (5.96 g, 19.7 mmol). To a solution of the ethyl ester (2.85 g, 9.44 mmol) in THF (20 mL) was added $LiBH_4$ (519 mg, 23.6 mmol) in three portions at 0 °C. The mixture was then stirred at room temperature for 10 h. The reaction was quenched by the addition of 0.2 N HCl, and the mixture was extracted with EtOAc. The collected EtOAc layer was washed with brine, dried over Na_2SO_4, and concentrated to give the crude alcohol. To a solution of this alcohol in pyridine (7.0 mL) was added Ac_2O (5.0 mL) at 0 °C, and the mixture was stirred for 30 min at 0 °C. The reaction was quenched by the addition of H_2O, and the mixture was concentrated. The residue was purified by column chromatography on Wakogel C-200 using hexane and increasing amounts of EtOAc to give **7** (3.11 g, 10.3 mmol, 99% in four steps). Compound **7**: ^{1}H NMR δ (500 MHz, 300 K, $CDCl_3$, 0.062 M): 2.05 (2H, m, ArCH_2CH_2-), 2.08 (3H, s, *Ac*), 2.99 (2H, m, ArCH_2-), 4.18 (2H, t, J = 6.2 Hz, $-CH_2$Ac), 7.23 (1H, t, J = 8.1 Hz, *Ar*), 7.73 (1H, dd, J = 8.1, 1.0 Hz, *Ar*), 7.80 (1H, dd, J = 8.0, 0.9 Hz, *Ar*); HRMS−FAB *m*/*z*: 302.0081 (MH^+, calcd for $C_{11}H_{13}NO_4Br$, 302.0027).

To a mixture of **7** (3.10 g, 10.3 mmol), $PdCl_2(PPh_3)_2$ (145 mg, 0.207 mmol), 1-octyne (1.80 mL, 12.2 mmol) and triethylamine (10 mL) was added CuI (78.0 mg, 0.411 mmol) at room temperature under an Ar atmosphere. The reaction mixture was stirred for 18 h at 50 °C and then filtered. The filtrate was concentrated and purified by column chromatography on Wakogel C-200 using hexane and increasing amounts of EtOAc to give **8** (1.92 g, 5.8 mmol, 56%). Compound **8**: ^{1}H NMR δ (500 MHz, 300 K, $CDCl_3$, 0.077 M): 0.91 (3H, t, J = 7.0 Hz, *octynyl*), 1.31−1.34 (4H, m, *octynyl*), 1.46 (2H, m, *octynyl*), 1.62 (2H, m, *octynyl*), 2.05 (2H, m, ArCH_2CH_2-), 2.06 (3H, s, *Ac*), 2.46 (2H, t, J = 7.1 Hz, *octynyl*), 3.04 (2H, m, ArCH_2-), 4.15 (2H, t, J = 6.4 Hz, $-CH_2$OAc), 7.26 (1H, t, J = 8.0 Hz, *Ar*), 7.60 (1H, dd, J = 8.0, 0.9 Hz, *Ar*), 7.71 (1H, dd, J = 8.0, 0.9 Hz, *Ar*); HRMS−FAB *m*/*z*: 332.1863 (MH^+, calcd for $C_{19}H_{26}NO_4$, 332.1862).

A mixture of **8** (1.92 g, 5.80 mmol), 10% Pd-C (192 mg) in EtOH (13 mL) was stirred vigorously under 1 atm of H_2 at room temperature for 2 h. The reaction mixture was filtered and then concentrated to give a crude amine. To a solution of the amine in THF (6.8 mL) was added acetic formic anhydride (2.6 mL) at 0 °C. The mixture was stirred at 0 °C for 1 h and concentrated. The residue was poured into saturated aqueous K_2CO_3 and extracted with EtOAc. The collected EtOAc layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on Wakogel C-200 using hexane and increasing amounts of EtOAc to give **9** (1.91 g, 5.74 mmol, 99% in two steps), in which two conformers existed in a ratio of 1:0.4. Compound **9**: ^{1}H NMR δ (500 MHz, 300 K, $CDCl_3$, 0.077 M) for the major conformer: 0.88 (3H, t, J = 6.9 Hz, *octyl*), 1.27−1.31 (10H, m, *octyl*), 1.56 (2H, m, *octyl*), 1.85 (2H, m, ArCH_2CH_2-), 2.14 (3H, s, *Ac*), 2.61 (2H, t, J = 7.5 Hz, *octyl*), 2.74 (2H, t, J = 7.9 Hz, ArCH_2-), 4.11 (2H, t, J = 6.0 Hz, $-CH_2$OAc), 7.00 (1H, d, J = 7.7 Hz, Ar), 7.09 (1H, d, J = 7.0 Hz, Ar), 7.17 (1H, t, J = 7.8 Hz, Ar), 7.91 (1H, br.d, J = 11.0 Hz, $-NHCHO$), 8.49 (1H, d, J = 11.0 Hz, $-NHCHO$); HRMS−FAB *m*/*z*: 333.2308 (M^+, calcd for $C_{20}H_{31}NO_3$, 333.2304).

To a solution of **9** (1.91 g, 5.74 mmol) in THF (30 mL) was added dropwise 1.0 M BH_3 in THF solution (17 mL) at 0 °C, and the reaction mixture was stirred for 2 h at 0 °C. The reaction was quenched by the addition of 10% aqueous citric acid (20 mL), and the mixture was extracted with EtOAc. The collected EtOAc layer was washed with brine, dried over Na_2SO_4, and then concentrated

to give the crude *N*-methylaniline. To a solution of the *N*-methylaniline in MeOH (20 mL) was added 1 N NaOH (5.0 mL). The reaction mixture was stirred at room temperature for 1 h and then concentrated. The residue was poured into H_2O, and the mixture was extracted with EtOAc. The collected EtOAc layer was washed with brine, dried over Na_2SO_4, and then concentrated. The residue was purified by column chromatography on Wakogel C-200 using hexane and increasing amounts of EtOAc to give **10** (1.59 g, 5.74 mmol, 99% in two steps). Compound **10**: ^{1}H NMR δ (500 MHz, 300 K, $CDCl_3$, 0.077 M): 0.88 (3H, t, J = 6.9 Hz, *octyl*), 1.22−1.34 (10H, m, *octyl*), 1.55 (2H, m, *octyl*), 1.77 (2H, m, $ArCH_2CH_2$−), 2.57 (2H, m, *octyl*), 2.66 (2H, t, J = 7.6 Hz, $ArCH_2$−), 2.84 (3H, s, *N*-CH_3), 3.64 (2H, t, J = 5.8 Hz, −CH_2OH), 6.53 (1H, dd, J = 8.0, 0.8 Hz, *Ar*), 6.62 (1H, dd, J = 7.9, 0.9 Hz, *Ar*), 7.09 (1H, t, J = 7.9 Hz, *Ar*); HR−EIMS *m*/*z*: 277.2403 (M^+, calcd for $C_{18}H_{31}NO$, 277.2406).

A mixture of **10** (1.56 g, 5.63 mmol), 2,6-lutidine (1.3 mL, 11.2 mmol), and Val-Tf [22] (2.26 g, 6.65 mmol) in 1,2-dichloroethane (20 mL) was refluxed at 80 °C for 16 h and then concentrated. The residue was purified by column chromatography on Wakogel C-200 using hexane and increasing amounts of EtOAc to stereospecifically give **11** (1.93 g, 4.13 mmol, 73%). Compound **11**: $[\alpha]_D$ −16.5° (c = 0.73, MeOH, 30.9 °C); ^{1}H NMR δ (500 MHz, 300 K, $CDCl_3$, 0.073 M): 0.89 (3H, t, J = 7.3 Hz, *octyl*), 0.91 (3H, d, J = 6.6 Hz, −$CH(CH_3)_2$), 1.18 (3H, d, J = 6.7 Hz, −$CH(CH_3)_2$), 1.27−1.39 (10H, m, *octyl*), 1.55 (2H, m, *octyl*), 1.70 (1H, m, $ArCH_2CH_2$), 1.82 (1H, m, $ArCH_2CH_2$), 2.25 (1H, m, −$CH(CH_3)_2$), 2.54 (1H, m, $ArCH_2$), 2.67 (2H, m, $ArCH_2$), 2.74 (1H, dd, J = 7.9, 4.7 Hz, O*H*), 2.84 (3H, s, *N*-CH_3), 2.89 (1H, m, $ArCH_2$), 3.21 (1H, d, J = 10.4 Hz, *N*-*CH*(*i*-Pro)CO_2Bn), 3.39 (1H, m, CH_2OH), 3.57 (1H, m, CH_2OH), 4.92 (2H, s, −CO_2CH_2Ph), 6.91 (1H, dd, J = 7.1, 2.2 Hz, *Ar*), 6.97 (2H, m, *Ar*), 7.02 (2H, m, *Ar*), 7.26 (3H, m, *Ar*); HR−EIMS *m*/*z*: 467.3403 (M^+, calcd for $C_{30}H_{45}NO_3$, 467.3399).

To a solution of **11** (1.93 g, 4.13 mmol) in CH_2Cl_2 (20 mL) was added the Dess−Martin reagent (2.07 g, 4.88 mmol) at room temperature under an Ar atmosphere. The reaction mixture was stirred for 30 min and then concentrated. The residue was poured into saturated aqueous Na_2SO_4 and extracted with EtOAc. The collected EtOAc layer was washed with brine, dried over Na_2SO_4, and then concentrated. The residue was purified by column chromatography on Wakogel C-200 using hexane and increasing amounts of EtOAc to give an aldehyde. A mixture of the aldehyde and (*R*)-phenylglycinol (518 mg, 3.78 mmol) in MeOH (6.3 mL) was stirred at room temperature for 30 min before it was heated to 40 °C. At this temperature, trimethylsilyl cyanide (0.687 mL, 5.16 mmol) was added dropwise, and the mixture was stirred for a further 1 h at the same temperature. The reaction mixture was poured into brine, and the mixture was extracted with EtOAc. The collected EtOAc layer was washed with brine, dried over Na_2SO_4, and then concentrated. The residue was purified by column chromatography on Wakogel C-200 using hexane and increasing amounts of EtOAc to give a diasteriomeric mixture of amino nitriles. A solution of the diastereomeric mixture in MeOH (20 mL) was refluxed at 80 °C for 3 h, and then concentrated. The residue was purified by column chromatography on Wakogel C-200 using hexane and increasing amounts of EtOAc to give (*S*,*S*) isomer **12** as a single diastereomer (1.79 g, 2.93 mmol, 85% in two steps). Compound **12**: $[\alpha]_D$ +51.5° (c = 0.20, MeOH, 30.9 °C); ^{1}H NMR δ (500 MHz, 300 K, $CDCl_3$, 0.041 M): 0.87 (3H, d, J = 6.6 Hz, −$CH(CH_3)_2$), 0.88 (3H, t, J = 7.1 Hz, *octyl*), 1.03 (3H, d, J = 6.7 Hz, −$CH(CH_3)_2$), 1.26−1.38 (10H, m, *octyl*), 1.55 (2H, m, *octyl*), 1.75 (2H, m, $ArCH_2CH_2$−), 2.05 (1H, m, −$CH(CH_3)_2$), 2.21 (2H, m, N*H*, O*H*), 2.57 (2H, m, *octyl*), 2.67 (1H, dt, J = 12.3, 4.3 Hz, $ArCH_2$−), 2.72 (3H, s, *N*-CH_3), 2.89 (1H, dt, J = 12.3, 5.1 Hz, $ArCH_2$−), 3.14 (1H, d, J = 9.7 Hz, *N*-*CH*(*i*-Pro)CO_2Bn), 3.30 (1H, t, J = 6.8 Hz, *N*-*CH*(Ph)CH_2OH), 3.58 (1H, t, J = 10.0 Hz, −CH_2-OH), 3.79 (1H, dd, J = 10.0, 4.0, −CH_2OH), 4.13 (1H, dd, J = 9.1, 4.0 Hz, *N*-*CH*(CN)CH_2−), 4.96 (2H, ABq, J = 12.3 Hz, CO_2CH_2Ph), 6.87 (1H, dd, J = 7.6, 1.4 Hz, *Ar*), 6.94 (1H, dd, J = 7.6, 1.4 Hz, *Ar*), 6.98 (1H, t, J = 7.6 Hz, *Ar*), 7.08 (2H, m, *Ar*), 7.26−7.35 (8H, m, *Ar*); HRMS−FAB *m*/*z*: 612.4204 (MH^+, calcd for $C_{39}H_{54}N_3O_3$, 612.4165).

A solution of **12** (1.40 g, 2.29 mmol) in HCl-saturated MeOH (25 mL) was stirred at room temperature for 36 h. The reaction was quenched by neutralization with cool 1N NaOH. The mixture was poured into brine (30 mL) and extracted with EtOAc. The collected EtOAc layer was washed with brine, dried over Na_2SO_4, and then concentrated. The residue was purified by column chromatography on Wakogel C-200 using hexane and increasing amounts of EtOAc to give **13** (860 mg, 1.34 mmol, 59%). Compound **13**: $[\alpha]_D$ −44.2° (c = 0.31, MeOH, 30.9 °C); ^{1}H NMR δ (500 MHz, 300 K, $CDCl_3$, 0.036 M): 0.87 (3H, d, J = 6.8 Hz, −$CH(CH_3)_2$), 0.88 (3H, t, J = 6.8 Hz, *octyl*), 1.08 (3H, d, J = 6.7 Hz, −$CH(CH_3)_2$), 1.26−1.34 (10H, m, *octyl*), 1.52 (2H, m, *octyl*), 1.61 (1H, br.s, O*H*), 1.69 (1H, m, $ArCH_2CH_2$−), 1.82 (1H, m, $ArCH_2CH_2$−), 2.11 (1H, br.s, N*H*), 2.20 (1H, m, −$CH(CH_3)_2$), 2.49 (1H, dt, J = 12.5, 3.5 Hz, $ArCH_2$−), 2.50 (2H, m, *octyl*), 2.75 (3H, s, *N*-CH_3), 2.92 (1H, dt, J = 12.5, 5.1 Hz, $ArCH_2$−), 3.16 (1H, d, J = 9.9 Hz, *N*-*CH*(*i*-Pro)CO_2Bn), 3.18 (1H, t, J = 6.7 Hz, *N*-*CH*(Ph)CH_2OH), 3.54 (1H, m, *N*-*CH*(CO_2CH_3)CH_2−), 3.69 (3H, s, −CO_2CH_3), 3.73 (2H, m, −CH_2OH), 4.97 (2H, ABq, J = 12.5 Hz, −CO_2CH_2Ph), 6.86 (1H, dd, J = 7.6, 1.5 Hz, *Ar*), 6.92 (1H, dd, J = 7.5, 1.5 Hz, *Ar*), 6.95 (1H, t, J = 7.5 Hz, *Ar*), 7.08 (2H, m, *Ar*), 7.23−7.36 (8H, m, *Ar*); HRMS−FAB *m*/*z*: 645.4256 (MH^+, calcd for $C_{40}H_{57}N_2O_5$, 645.4267).

A mixture of **13** (202 mg, 0.313 mmol) and 10% Pd-C (20 mg) in MeOH (5.0 mL) was stirred vigorously under 1 atm of H_2 at room temperature for 18 h. The reaction mixture was filtered and then concentrated to give the crude amino acid. To a solution of the amino acid in DMF (9.0 mL) was added diphenylphosphoryl azide (135 μL, 0.627 mmol) and triethylamine (131 μL, 0.942 mmol) at 0 °C. The solution was stirred at room temperature for 27 h. The reaction mixture was poured into H_2O and extracted with EtOAc. The collected EtOAc layer was washed with brine, dried over Na_2SO_4, and then concentrated. The residue was purified by column chromatography on Wakogel C-200 using hexane and increasing amounts of EtOAc to give a lactam. To a solution of the lactam in THF (1.0 mL) was added $LiBH_4$ (5.0 mg, 0.228 mmol) at 0 °C, and the mixture was stirred at room temperature for 1 h. The reaction mixture was poured into H_2O, and the mixture was extracted with EtOAc. The EtOAc layer was washed with brine, dried over Na_2SO_4, and then concentrated. The residue was purified by HPLC on YMC-SH-342-5 using 85% MeOH to give **4** (24.7 mg, 64.0 μmol, 84%) in which two conformers existed in a ratio of 3.7:1.0. The purity of **4** was more than 95%, which was confirmed by two diverse HPLC systems on SH-342-5 using 85% MeOH (flow rate of 8.0 mL/min; retention time of 36.0 min) and 70% MeCN (flow rate of 8.0 mL/min; retention time of 40.7 min). Compound **4**: $[\alpha]_D$ +118.9° (c = 1.09, MeOH, 31.3 °C); UV λ_{max} (MeOH) nm (ϵ): 274 (2,600), 238 (3,000), 204 (21,900); ^{13}C NMR δ (125 MHz, 300 K, $CDCl_3$, 0.112 M): 14.12, 19.00, 19.69, 22.68, 23.80, 24.46, 29.27, 29.49, 29.91, 31.17, 31.88, 32.97, 35.26, 36.02, 54.49, 65.02, 76.14, 127.31, 128.82, 142.99, 143.43, 151.34, 170.42; ^{1}H NMR δ (500 MHz, 300 K, $CDCl_3$, 0.112 M) for the major conformer: 0.88 (3H, t, J = 6.7 Hz, *octyl*), 0.90 (3H, d, J = 6.3 Hz, −$CH(CH_3)_2$), 1.16 (3H, d, J = 6.6 Hz, −$CH(CH_3)_2$), 1.21−1.37 (10H, m, *octyl*), 1.50 (3H, m, *octyl*, $ArCH_2CH_2$−), 2.05 (1H, br.s, O*H*), 2.27 (2H, m, $ArCH_2CH_2$− $CH(CH_3)_2$), 2.34 (1H, dd, J = 14.8, 9.1 Hz, $ArCH_2$−), 2.47 (1H, m, *octyl*), 2.56 (1H, m, *octyl*), 2.71 (3H, s, *N*-CH_3), 2.76 (1H, dd, J = 14.8, 8.9 Hz, Ar−CH_2), 2.85 (1H, d, J = 10.9 Hz, *NCH*(*i*-Pr)CONH−), 3.40 (1H, m, −CH_2-OH), 3.57 (1H, m, −CH_2OH), 4.36 (1H, m, *NCH*(CH_2OH)CH_2−), 4.58 (1H, br.d, J = 10.6 Hz, N*H*), 6.96 (1H, d, J = 7.6 Hz, Ar), 7.02 (1H, d, J = 6.7 Hz, Ar), 7.09 (1H, t, J = 7.6 Hz, Ar); for the minor conformer: 1.12 (3H, d, J = 6.9 Hz, −$CH(CH_3)_2$), 1.19 (3H, d, J = 6.7 Hz, −$CH(CH_3)_2$), 1.88 (1H, dd, J = 13.5, 3.8 Hz, $ArCH_2CH_2$−), 2.61 (3H, s, *N*-CH_3), 2.76 (1H, m, $ArCH_2$−), 3.10 (1H, br.s, O*H*), 3.20 (1H, t, J = 12.8 Hz, $ArCH_2$−), 3.51 (2H, m, −CH_2OH), 3.70 (1H, d, J = 5.9 Hz, *NCH*(*i*-Pr)CONH−), 4.67 (1H, m, *NCH*(CH_2OH)CH_2−), 5.65 (1H, br.d, J = 10.2 Hz, N*H*), 6.96 (1H, m, Ar), 7.07 (1H, m, Ar), 7.13 (1H, d, J = 7.8 Hz, Ar). Other

peaks had weak intensities and overlapped those of the major conformer. HR−EIMS *m/z*: 388.3089 (M^+, calcd for $C_{24}H_{40}N_2O_2$, 388.3090).

Conformational Analysis of 8-Octyl-benzolactam-V9 (4). The main conformation of 8-octyl-benzolactam-V9 (**4**) was estimated by the Chem 3D (Cambridge Soft) and AMOSS-H11 (NEC quantum chemistry group) programs. The initial structures were calculated by molecular mechanics calculations using the MM2 theory with the distance between two protons (H-2 and H-4, and H-7β and H-16) fixed at 2 Å, congruent with NOE data. The resultant structures were optimized by a semiempirical quantum mechanics calculation using the PM3 theory. Further optimization of these calculated structures was carried out by ab initio molecular orbital schemes using a Hartree−Fock theory with the 6-31G* basis set to give the most stable conformation.

A conformation search was carried out using the random search method implemented in Sybyl 7.1 (Tripos, Inc.). The octyl group of **4** was replaced with the ethyl group to simplify the calculation. The following conditions and parameters were used: all rotatable bonds were perturbed; the energy cut off was set to 15 kcal/mol; the maximum number of search iterations and the maximum number of hits were both set to 100 000; and the RMS threshold was set to 0.2 Å. The conformations produced by the random conformational search were fully optimized using the MMFF94s force field. The dielectric constant (ϵ) was set to 5.0 ($CHCl_3$). Powell's method was used for energy minimization until the gradient value was smaller than 0.001 kcal/mol·Å. As a result, we obtained 244 conformations with energy values within 10 kcal/mol from the global minimum, and they were classified into 11 groups (sofa, *r-trans*-fold-like, fold, *r-trans*-fold, *cis*-sofa, *trans*-fold-like, *r*-sofa, *r-cis*-sofa, *cis*-sofa-like, *r*-fold, and twist) on the basis of Itai's nomenclature (Supporting Information).[24] The global minimum (sofa) was identical to that of the major conformer of **4** (Figure 4).

Inhibition of Specific [^{3}H]PDBu Binding to PKC Isozyme C1 Peptides. The [^{3}H]PDBu binding to the PKC isozyme C1 peptides was evaluated using the procedure of Sharkey and Blumberg[24] with modifications, as reported previously,[14] under the following conditions: 50 mM Tris-maleate buffer (pH 7.4 at 4 °C), 5−20 nM a PKC isozyme C1 peptide, 20−40 nM [^{3}H]PDBu (19.0 Ci/mmol), 50 μg/mL 1,2-di(*cis*-9-octadecenoyl)-*sn*-glycero-3-phospho-L-serine, 3 mg/mL bovine γ-globulin, and various concentrations of 8-octyl-benzolactam-V9 (**4**). The binding affinity was evaluated by the concentration required to cause 50% inhibition of the specific [^{3}H]-PDBu binding, IC_{50}, which was calculated by a computer program (SAS) with a probit procedure. The binding constant, K_i, was calculated using the method of Sharkey and Blumberg.[24]

Translocation and Activation of PKC Isozymes. HeLa cells transfected with GFP-tagged PKC isozymes or GFP-tagged PKC isozymes and tandem DsRed2-tagged MARCKS were cultured for 16−48 h for maximal fluorescence expression. The media was then replaced with the normal HEPES buffer composed of 135 mM NaCl, 5.4 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, 5 mM 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES), and 10 mM glucose at pH 7.3. The translocation of the GFP-tagged PKCs was triggered by the addition of the various compounds to the HEPES buffer to obtain the appropriate final concentration. All experiments were performed at 37 °C. The fluorescence of GFP and DsRed2 was monitored by confocal laser scanning fluorescent microscopy (Carl Zeiss, Jena, Germany) at 488 nm argon excitation with a 505−550 nm band-pass barrier filter for GFP and at 543 nm HeNe excitation with a 560 nm long-pass barrier filter.

Quantitative Analysis of Membrane Translocation of PKCs. To quantitatively determine the translocation to the plasma membrane, the time series of confocal fluorescence images of cells expressing GFP-tagged PKCs were recorded before and after the stimulation of each compound. The relative plasma membrane fluorescence intensity was determined in each image using line intensity profiles across each one of the cells. Relative plasma membrane localization (R) was defined as $R = I_{pm}/I_{cyt}$, where I_{pm} and I_{cyt} are the plasma membrane fluorescence intensity and the average cytosolic fluorescence intensity, respectively. After each compound was added at various concentrations, the R values were plotted against time. The resulting curves represent the time course of plasma membrane translocation of GFP-tagged PKC subtypes of each compound (Supporting Information). The maximum R value represents the maximum membrane translocation of each PKC in response to the compound at various concentrations and are summarized in Table 2.

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Supporting Information Available: Translocation of all GFP-tagged PKC isozymes induced by 1 μM of ILV and **3** and 10 μM of **4** in HeLa cells along with NMR and conformational analyses of **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Nishizuka, Y. The role of protein kinase C in cell surface signal transduction and tumour promotion. *Nature* **1984**, *308*, 693−698.
(2) Nishizuka, Y. Protein kinase C and lipid signaling for sustained cellular responses. *FASEB J.* **1995**, *9*, 484−496.
(3) Fujiki, H.; Sugimura, T. New classes of tumor promoters: teleocidin, aplysiatoxin, and palytoxin. *Adv. Cancer Res.* **1987**, *49*, 223−264.
(4) 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.
(5) Endo, Y.; Shudo, K.; Okamoto, T. Molecular requirements for epigenetic modulators. Synthesis of active fragments of teleocidins and lyngbyatoxin. *Chem. Pharm. Bull.* **1982**, *30*, 3457−3460.
(6) Irie, K.; Hirota, M.; Hagiwara, N.; Koshimizu, K.; Hayashi, H.; Murao, S.; Tokuda, H.; Ito, Y. The Epstein-Barr virus early antigen inducing indole alkaloids, (−)-indolactam V and its related compounds produced by Actinomycetes. *Agric. Biol. Chem.* **1984**, *48*, 1269−1274.
(7) Kazanietz, M. G.; Areces, L. B.; Bahador, A.; Mischak, H.; Goodnight, J.; Mushinski, J. F.; Blumberg, P. M. Characterization of ligand and substrate specificity for the calcium-dependent and calcium-independent protein kinase C isozymes. *Mol. Pharmacol.* **1993**, *44*, 298−307.
(8) Hurley, J. H.; Newton, A. C.; Parker, P. J.; Blumberg, P. M.; Nishizuka, Y. Taxonomy and functions of C1 protein kinase C homology domains. *Protein Sci.* **1997**, *6*, 477−480.
(9) Reddig, P. J.; Dreckschimdt, 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.
(10) Chida, K.; Hara, T.; Hirai, T.; Konishi, C.; Nakamura, K.; Nakao, K.; Aiba, A.; Katsuki, M.; Kuroki, T. Disruption of protein kinase Cη results in impairment of wound healing and enhancement of tumor formation of mouse skin carcinogenesis. *Cancer Res.* **2003**, *63*, 2404−2408.
(11) Reddig, P. J.; Dreckschmidt, N. E.; Zou, J.; Bourguignon, S. E.; Oberley, T. D.; Verma, A. K. Transgenic mice overexpressing protein kinase Cϵ in their epidermis exhibit reduced papilloma burden but enhanced carcinoma formation after tumor promotion. *Cancer Res.* **2000**, *60*, 595−602.
(12) Szallasi, Z.; Bögi, K.; Gohari, S.; Biro, T.; Acs, P.; Blumberg, P. M. Non-equivalent roles for the first and second zinc fingers of protein kinase Cδ. *J. Biol. Chem.* **1996**, *271*, 18299−18301.
(13) Hunn, M.; Quest, A. F. G. Cysteine-rich regions of protein kinase Cδ are functionally non-equivalent. *FEBS Lett.* **1997**, *271*, 18299−18301.
(14) 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.

(15) Irie, K.; Oie, K.; Nakahara, A.; Yanai, Y.; Ohigashi, H.; Wender, P. A.; Satomi, Y.; Nishino, H. 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.
(16) Irie, K.; Nakahara, A.; Nakagawa, Y.; Ohigashi, H.; Shindo, M.; Fukuda, H.; Konishi, H.; Kikkawa, U.; Kashiwagi, K.; Saito, N. Establishment of a binding assay for protein kinase C isozymes using synthetic C1 peptides and development of new medicinal leads with protein kinase C isozymes and C1 domain selectivity. *Pharmacol. Ther*. **2002**, *93*, 271−281.
(17) Nakagawa, Y.; Irie, K.; Komiya, Y.; Ohigashi, H.; Tsuda, K. Synthesis, conformation and PKC isozyme surrogate binding of indolinelactam-Vs, new conformationally restricted analogues of (−)-indolactam-V. *Tetrahedron* **2004**, *60*, 7077−7084.
(18) Irie, K.; Nakagawa, Y.; Ohigashi, H. Toward the development of new medicinal leads with selectivity for protein kinase C isozymes. *Chem. Rec*. **2005**, *5*, 185−195.
(19) Irie, K.; Isaka, T.; Iwata, Y.; Yanai, Y.; Nakamura, Y.; Koizumi, F.; Ohigashi, H.; Wender, P. A.; Satomi, Y.; Nishino, H. Synthesis and biological activities of new conformationally restricted analogues of (−)-indolactam-V: elucidation of the biologically active conformation of the tumor-promoting teleocidins. *J. Am. Chem. Soc*. **1996**, *118*, 10733−10743.
(20) Endo, Y.; Ohno, M.; Hirano, M.; Itai, A.; Shudo, K. Synthesis, conformation, and biological activity of teleocidin mimics, benzolactams. A clarification of the conformational flexibility problem in structure−activity studies of teleocidins. *J. Am. Chem. Soc*. **1996**, *118*, 1841−1855.
(21) Thorand, S.; Krause, N. Improved procedures for the palladium-catalyzed coupling of terminal alkynes with aryl bromides (Sonogashira coupling). *J. Org. Chem*. **1998**, *63*, 8551−8553.
(22) Kogan, T. P.: Somers, T. C.; Venuti, M. C. A regio- and stereocontrolled total synthesis of (−)-indolactam-V. *Tetrahedron* **1990**, *46*, 6623−6632.
(23) Chakraborty, T. K.; Hussain, K. A.; Reddy, G. V. α-Phenylglycinol as chiral auxiliary in diastereoselective strecker synthesis of α-amino acids. *Tetrahedron* **1995**, *51*, 9179−9190.
(24) Kawai, T.; Ichinose, T.; Takeda, M.; Tomioka, N.; Endo, Y.; Yamaguchi, K.; Shudo, K.; Itai, A. Prediction of ring conformations of indolactams. Crystal and solution structures. *J. Org. Chem*. **1992**, *57*, 6150−6155.
(25) 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.
(26) Kraft, A. S.; Anderson, W. B.; Cooper, H. L.; Sando, J. J. Decrease in cytosolic calcium/phospholipid-dependent protein kinase activity following phorbol ester treatment of ELA4 thymoma cells. *J. Biol. Chem*. **1982**, *257*, 13193−13196.
(27) Wada, R.; Suto, Y.; Kanai, M.; Shibasaki, M. Dramatic switching of protein kinase C agonist/antagonist activity by modifying the 12-ester side chain of phorbol esters. *J. Am. Chem. Soc*. **2002**, *124*, 10658−10659.
(28) Sakai, N.; Sasaki, K.; Ikegaki, N.; Shirai, Y.; Ono, Y.; Saito, N. Direct Visualization of the translocation of the γ-subspecies of protein kinase C in living cells using fusion proteins with green fluorescent protein. *J. Cell Biol*. **1997**, *139*, 1465−1476.
(29) Oancea, E.; Meyer, T. Protein kinase C as a machine for decoding calcium and diacylglycerol signals. *Cell* **1998**, *95*, 307−318.
(30) Wang, Q. J.; Bhattacharyya, D.; Garfield, S.; Nacro, K.; Marquez, V. E.; Blumberg, P. M. Differential localization of protein kinase C δ by phorbol esters and related compounds using a fusion protein with green fluorescent protein. *J. Biol. Chem*. **1999**, *274*, 37233−37239.
(31) Ohmori, S.; Sakai, N.; Shirai, Y.; Yamamoto, H.; Miyamoto, E.; Shimizu, H.; Saito, N. Importance of protein kinase C targeting for the phosphorylation of its substrate, myristoylated alanine-rich C-kinase substrate. *J. Biol. Chem*. **2000**, *275*, 26449−26457.
(32) Nakagawa, Y.; Irie, K.; Yanagita, R. C.; Ohigashi, H.; Tsuda, K. Indolactam-V is involved in the CH/π interaction with Pro-11 of the PKCδ C1B domain: application for the structural optimization of the PKCδ ligand. *J. Am. Chem. Soc*. **2005**, *127*, 5746−5747.
(33) Garcia-Bermejo, M. L.; Leskow, F. C.; Fujii, T.; Wang, Q.; Blumberg, P. M.; Ohba, M.; Kuroki, T.; Han, K.-C.; Lee, J.; Marquez, V. E.; Kazanietz, M. G. Diacylglycerol (DAG)-lactones, a new class of protein kinase C (PKC) agonists, induce apoptosis in LNCaP prostate cancer cells by selective activation of PKCα. *J. Biol. Chem*. **2002**, *277*, 645−655.

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