Synthesis, Conformational Analysis, and Biological Evaluation of 1-Hexylindolactam-V10 as a Selective Activator for Novel Protein Kinase C Isozymes

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Conventional and novel protein kinase C (PKC) isozymes are the main targets of tumor promoters. We developed 1-hexylindolactam-V10 (**5**) as a selective activator for novel PKC isozymes that play important roles in various cellular processes related to tumor promotion, ischemia−reperfusion injury in the heart, and Alzheimer's disease. The compound existed as a mixture of three conformers. The *trans*-amide restricted analogues of **5** (**14** and **15**) hardly bound to PKC isozymes, suggesting that the active conformation of **5** could be that with a *cis*-amide. Compound **5** selectively translocated novel PKC isozymes over conventional PKC isozymes in HeLa cells at 0.1–1 μM. These results suggest that **5** could be useful for the functional analysis of novel PKC isozymes.

Introduction

Protein kinase C (PKC[a])isozymes comprise a family of serine/threonine kinases in intracellular signal transduction cascades and are involved in many cellular events such as differentiation, proliferation, and apoptosis.[1,2] The PKC family consists of at least 10 isozymes and is subdivided into three classes based on their second messenger requirements (Figure 1):[3] conventional PKC isozymes (cPKCs α, βI, βII, γ), novel PKC isozymes (nPKCs δ, ϵ, η, θ), and atypical PKC isozymes (aPKCs ζ, λ/ι). Both cPKCs and nPKCs possess two C1 domains (C1A and C1B) in the regulatory domain, which bind the second messenger 1,2-*sn*-diacylglycerol (DAG). The binding of calcium ion to the C2 domains also causes activation of cPKCs, while the C2-like domains in nPKCs lack the ability to bind calcium ion. aPKCs bind neither DAG nor calcium.

PKCs have also attracted much attention as main targets of tumor promoters such as phorbol esters, aplysiatoxins, and teleocidins.[4] Although the precise mechanism of tumor promotion through PKCs is not well understood,[5] recent studies revealed the involvement of several PKC isozymes in carcinogenesis. For example, antisense depletion of cPKCs (PKCα or βI) in human gastric cancer cells reduced the size and rate of formation of tumors in mouse xenografts.[6] Whereas overexpression of PKCϵ in various cell lines resulted in a tumorigenic phenotype,[7,8] PKCδ produced antiproliferative and apoptotic signals in several cell types.[9] Depletion of PKCδ in Src-overexpressing cells by prolonged phorbol ester treatment gave a malignant phenotype,[10] and transgenic mice overexpressing PKCδ in the epidermis were resistant to skin tumor promotion by 12-*O*-tetradecanoylphorbol 13-acetate,[11] indicating that PKCδ plays a tumor suppressor role. Moreover, knockout of PKCη caused enhancement of tumor promotion.[12]

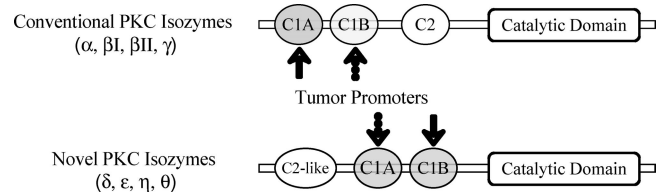

Figure 1. Protein kinase C (PKC) isozymes. Solid arrows indicate main tumor promoter-binding domains.

To analyze the role of each PKC isozyme in tumorigenesis and to develop a practical tumor suppressor, isozyme-selective PKC ligands are strongly needed. Recently, the selective PKCβ inhibitor enzastaurin (LY317615) was shown to be a suppressor of proliferation for several types of cancer cells.[13,14] However, ligands selective for nPKCs in the submicromolar range have not yet been developed. nPKCs play pivotal roles in various cellular mechanisms other than tumor promotion. For example, PKCϵ is involved in mediating ischemic preconditioning-induced cardioprotection from ischemia−reperfusion injury, whereas inhibition of PKCδ during reperfusion protects the heart from reperfusion-induced damage.[15] PKCϵ is also related to nociceptive processing in primary afferents[15] and plays a protective role in the development of Alzheimer disease.[16] Furthermore, PKCϵ could be involved in the dynamic regulation of the glucose-induced release of insulin in pancreatic β-cells.[17] Novel PKC-selective activators would thus be useful for the analysis of these cellular responses and as potential therapeutic leads.

Indolactam-V (**1**, Figure 2),[18,19] the core structure of teleocidins, is a superior lead compound for the development of agents with selectivity for nPKCs because of its simple structure and ease of derivatization. Kozikowski and colleagues[20] reported that 7-decylbenzolactam-V8 (**6**) showed some selectivity for novel PKCs (PKCδ and ϵ). Our molecular design based on the indolinelactam-V compounds[21] produced 8-octylbenzolactam-V9 (**2**),[22] showing marked selectivity for binding isolated C1B domains of nPKCs. Compound **2** selectively translocated PKCϵ and PKCη to the plasma membrane to activate them in HeLa cells. However, the concentration required to induce the

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[a]Abbreviations: PKC, protein kinase C; Boc-ON, 2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile; cPKC, conventional protein kinase C isozyme; nPKC, novel protein kinase C isozyme; aPKC, atypical protein kinase C isozyme; DAG, 1,2-*sn*-diacylglycerol; dfP, 4,4-difluoro-L-proline; GFP, green fluorescent protein; MARCKS, myristoylated alanine-rich C kinase substrate; PDBu, phorbol 12,13-dibutyrate; TPS, 2,4,6-triisopropylbenzenesulfonyl hydrazide.

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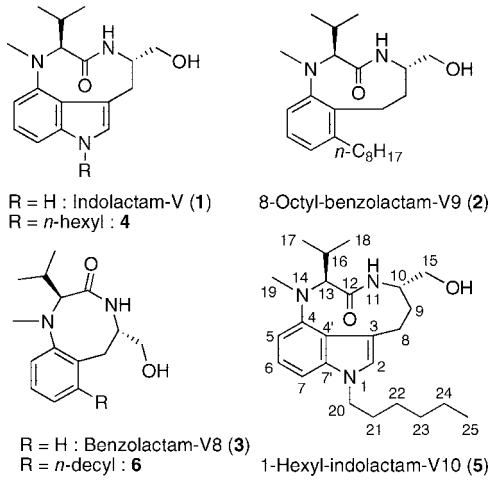


Figure 2. Structures of indolactam-V (**1**) and its derivatives (**2**–**6**).

Table 1. K_i Values for Inhibition of the Specific Binding of [^{3}H]PDBu by Indolactam-V (**1**), 8-Octylbenzolactam-V9 (**2**), 1-Hexylindolactam-V (**4**), and 1-Hexylindolactam-V10 (**5**)

PKC C1 peptides	K_i (nM)				K_d (nM) PDBu
	1[a]	**2**[b]	**4**[c]	**5**	
α-C1A	21 (1.2)[d]	>10000	5.8 (0.1)	6700 (990)	1.1
α-C1B	4000 (870)	>10000	43 (10)	>10000	5.3
β-C1A	19 (4.5)	>10000	9.8 (1.6)	9200 (1600)	1.3
β-C1B	140 (44)	>10000	11 (0.8)	440 (24)	1.3
γ-C1A	89 (3.9)	>10000	15 (3.5)	11000 (300)	1.5
γ-C1B	59 (8.0)	>10000	39 (6.9)	420 (20)	1.2
δ-C1B	11 (0.6)	220 (9.0)	0.46 (0.07)	15 (1.3)	0.53
ϵ-C1B	7.7 (1.2)	510 (39)	0.47 (0.12)	29 (2.8)	0.81
η-C1B	5.5 (0.6)	140 (13)	0.34 (0.11)	25 (2.3)	0.45
θ-C1B	8.7 (1.2)	290 (15)	1.4 (0.03)	12 (1.5)	0.72

[a] Cited from ref 44. [b] Cited from ref 22. [c] Cited from ref 21. [d] Standard deviation from at least two separate experiments.

translocation (10 μM) was at least 20 times higher than that of **1**. Recently, we suggested that the indole ring of **1** could be involved in the CH/π interaction (acidic CH-to-π interaction defined by Nishio)[23] with the hydrogen atom at position 4 of Pro-11 of the PKCδ C1B (δ-C1B) domain and that the weaker affinity of benzolactam-V8 (**3**),[24] the benzene ring analogue of **1**, might be due to the loss of this interaction.[25] CH/π interaction is a weak attractive force between CH groups and π-electron systems. The energy of the CH/π interaction involving nonactivating CH groups is very small (∼1.5 kcal/mol), although it plays important roles in proteins and molecular assemblies.[23] This means that the indole ring of indolactam analogues would be necessary for their marked affinity for PKCs. In fact, the 1-hexyl derivative of **1** (**4**) showed moderate selectivity for nPKC C1B domains with strong absolute affinity (Table 1).[21]

Since expansion of the ring of lactam in the benzolactam skeleton increased the selectivity for nPKCs as exemplified by **2**, we designed 1-hexylindolactam-V10 (**5**), a ring expansion analogue of 1-hexylindolactam-V (**4**) with the indole ring that might be involved in the CH/π interaction with the PKC C1 domains. This paper describes the synthesis, conformational analysis, and biological evaluation of **5**.

Results and Discussion

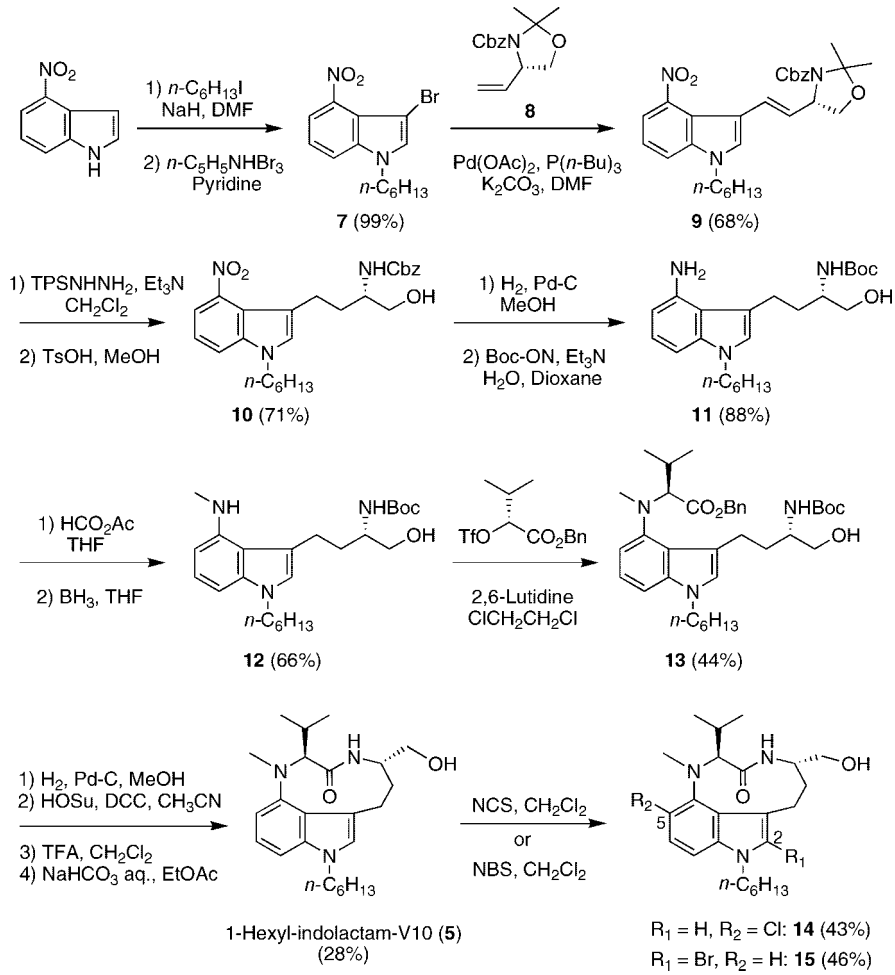
1-Hexylindolactam-V10 (**5**) was synthesized from 4-nitroindole as shown in Scheme 1. A hexyl group was introduced at position 1 of 4-nitroindole by an S_N2 reaction with 1-iodohexane, followed by bromination at position 3 to give **7** (99% in two steps). A Mizoroki−Heck reaction[26,27] of **7** with the amino alcohol unit (**8**)[28] gave **9** in 68% yield. Reduction of **9** using TPS diimide,[29] followed by deprotection of the acetonide moiety, afforded the amino alcohol **10** (71% in two steps). Reduction of the nitro group and deprotection of the Cbz group were accomplished simultaneously by catalytic hydrogenation to give the diamine whose aliphatic amino group was selectively protected with a Boc group using Boc-ON to give **11** (88%). Monomethylation of **11** using acetic formic anhydride followed by reduction gave **12** (66% in two steps). The valine unit was stereoselectively introduced by the S_N2 reaction using Kogan's triflate[30] to give **13** (44%). After deprotection of the benzyl group by catalytic hydrogenation, the resulting carboxylic acid was converted to the activated ester. Deprotection of the Boc group using TFA followed by cyclization gave **5** (28% in four steps). The overall yield was 3.4% (14 steps).

The ^{1}H NMR spectrum of **5** indicated that at room temperature in $CDCl_3$ (0.023 M), **5** existed as three conformers designated A, B, and C in a ratio of 8.5:1.7:1. The ratio of the conformers was dependent on the solvent and concentration. For example, it was 7.5:1.4:1 in $CDCl_3$ (0.050 M) and 8.5:2.6:1 in CD_3OD (0.046 M) (Supporting Information). All proton and carbon atoms in **5** were assigned in $CDCl_3$ using ^{1}H−^{1}H COSY and scalar heteronuclear experiments (HMBC and HMQC). A set of nonexchangeable protons almost coalesced at 70 °C in pyridine-d_5, and the spectrum of **5** did not change qualitatively in a variable-temperature NMR study at 300, 273, 253, and 243 K (Supporting Information), suggesting that the three sets of the signals could be assigned to each conformer.

Indolactam-V (**1**) and 1-hexylindolactam-V (**4**) exist in an equilibrium of the two conformers in solution,[21,31] the active twist form with a *cis*-amide and the inactive sofa form with a *trans*-amide.[32–35] Structural modification of **1** often changes the delicate conformation of the nine-membered lactam to abolish the binding to PKC of the resulting analogue.[21,34] To confirm whether **5** with a 10-membered lactam could adopt a conformation similar to the active twist form of **1**, a conformational analysis of **5** was performed on the basis of NMR studies. Conformer A of **5** was assigned as the sofalike form based on the upfield shift of H-11 (δ 5.27) and H-13 (δ 3.10) signals, considered to be due to the magnetic anisotropy of the indole ring and a significant NOE between these protons (Figure 3). To estimate conformers B and C of **5**, a search was carried out that provided eight conformers, which were similar to those of **1**[32] (Supporting Information). The global minimum conformer was identical to conformer A (the sofalike form). In conformer B, an upfield shift of the H-11 (δ 5.53) signal and a significant NOE between the H-11 and H-13 (δ 3.23) signals were observed as in conformer A. Unlike in the sofalike form, however, an upfield shift of the H-10 (δ 3.06) signal and an NOE between the H-5 (δ 7.08) and H-16 (δ 2.06) signals were observed in conformer B, suggesting that the conformer might be the *r-trans*-fold-like form, being the second most stable of the eight conformers obtained by the search. In conformer C, there was no significant NOE. However, downfield shifts of H-10 (δ 4.70), H-11 (δ 6.48), and H-13 (δ 4.69) signals and upfield shifts of aromatic H-5 (δ 6.48) and H-7 (δ 6.88) signals suggested that conformer C might be the twistlike form with a *cis*-amide that corresponds to the active conformations of **1** and **3**.[33,34]

The affinity of **5** for each C1 domain of PKC isozymes was evaluated on the basis of inhibition of the specific binding of [^{3}H]phorbol 12,13-dibutyrate (PDBu) to each PKC C1 peptide prepared by solid-phase peptide synthesis followed by zinc folding.[36,37] Since nPKC C1Bs are thought to be the main binding domains for tumor promoters,[38–40] the affinity of **5** for

Scheme 1. Synthesis of 1-Hexylindolactam-V10 (**5**) along with Its *trans*-Amide Restricted Analogues (**14** and **15**)



nPKCs was evaluated using the nPKC C1B peptides. The inhibition constants (K_i) calculated by the method of Blumberg and colleagues[41] are summarized in Table 1. Although the affinity of **5** for nPKC C1Bs was 1–2 orders of magnitude lower than that of **4**, the selectivity for nPKC C1Bs over cPKC C1As, the major binding sites for tumor promoters,[42,43] was significantly improved compared to **4**. In addition, the absolute binding affinity for nPKC C1Bs was comparable to that of **1**[44] and was 10–20 times stronger than that of **2**.

Recently, we showed the indole ring of **1** could be involved in the CH/π interaction with the hydrogen atom at position 4 of Pro-11 of PKCδ C1B domain.[25] Although the stabilization energy of the CH/π interaction is generally small ($\sim$1.5 kcal/mol), this weak interaction has been revealed to play important roles in the stability of proteins and ligand–receptor interaction. The photoactive yellow protein was shown to be stabilized by the CH/π interaction between the Lys CH group and the Phe residue.[45] Umezawa and colleagues successfully developed a protein−tyrosine phosphatase inhibitor using the concept of CH/π interaction.[46] Varnavas and colleagues synthesized the potent CCK_1 receptor agonist, the higher affinity of which could be explained by the CH/π interaction.[47] According to these reports, we postulated that the difference in PKC isozyme profile between **1** and **5** might be partially responsible for the CH/π interaction.

In order to examine the effects of the CH/π interaction on the binding to each PKC isozyme, we evaluated affinities of **1** and **3**−**5** for the P11dfP mutant of δ-C1B[25] and γ-C1A, where Pro-11 was replaced with 4,4-difluoro-L-proline (dfP). Because a fluorine atom has the second smallest van der Waals radius after and significantly greater electronegativity than a hydrogen atom, substitution with a fluorine atom could inhibit the CH/π interaction of the target hydrogen atom with minimum conformational change of the peptides.[48] In fact, little conformational change due to the fluorine mutation would occur because the K_i values of benzolactam-V8 (**3**), in which the indole ring of **1** is replaced with a benzene ring, were similar between δ-C1B and δ-C1B(P11dfP) and between γ-C1A and γ-C1A(P11dfP), as shown in Table 2.

K_i values of **1**, **4**, and **5** for these mutant peptides are also listed in Table 2. Compounds **4** and **5** showed 8 times less affinity for δ-C1B(P11dfP) than for wild-type δ-C1B, suggesting that **4** and **5** as well as **1** could be involved in the CH/π interaction with the hydrogen atom at position 4 of Pro-11 in δ-C1B. In contrast, the affinity of **5** for γ-C1A(P11dfP) was almost equal to that for wild-type γ-C1A, whereas **1** and **4** showed about 8 and 5 times less affinity for γ-C1A(P11dfP) than for wild-type γ-C1A, respectively. Although the ratio of K_i values of **5** for the wild-type and mutant γ-C1A might not be precise because of its weak affinities, the difference between the CH/π interaction of **1** and **5** with γ-C1A seems significant. These results suggest the weak CH/π interaction to be one of the reasons for the lower affinity of **5** for γ-C1A.

Previous studies indicated that the twist form with a *cis*-amide is the active conformation of **1**.[33–35] To enhance the absolute binding affinity of **5** to nPKCs, we attempted to increase the ratio of the twist form in **5** through the formation of a bridge between positions 5 and 19 or through introduction of an

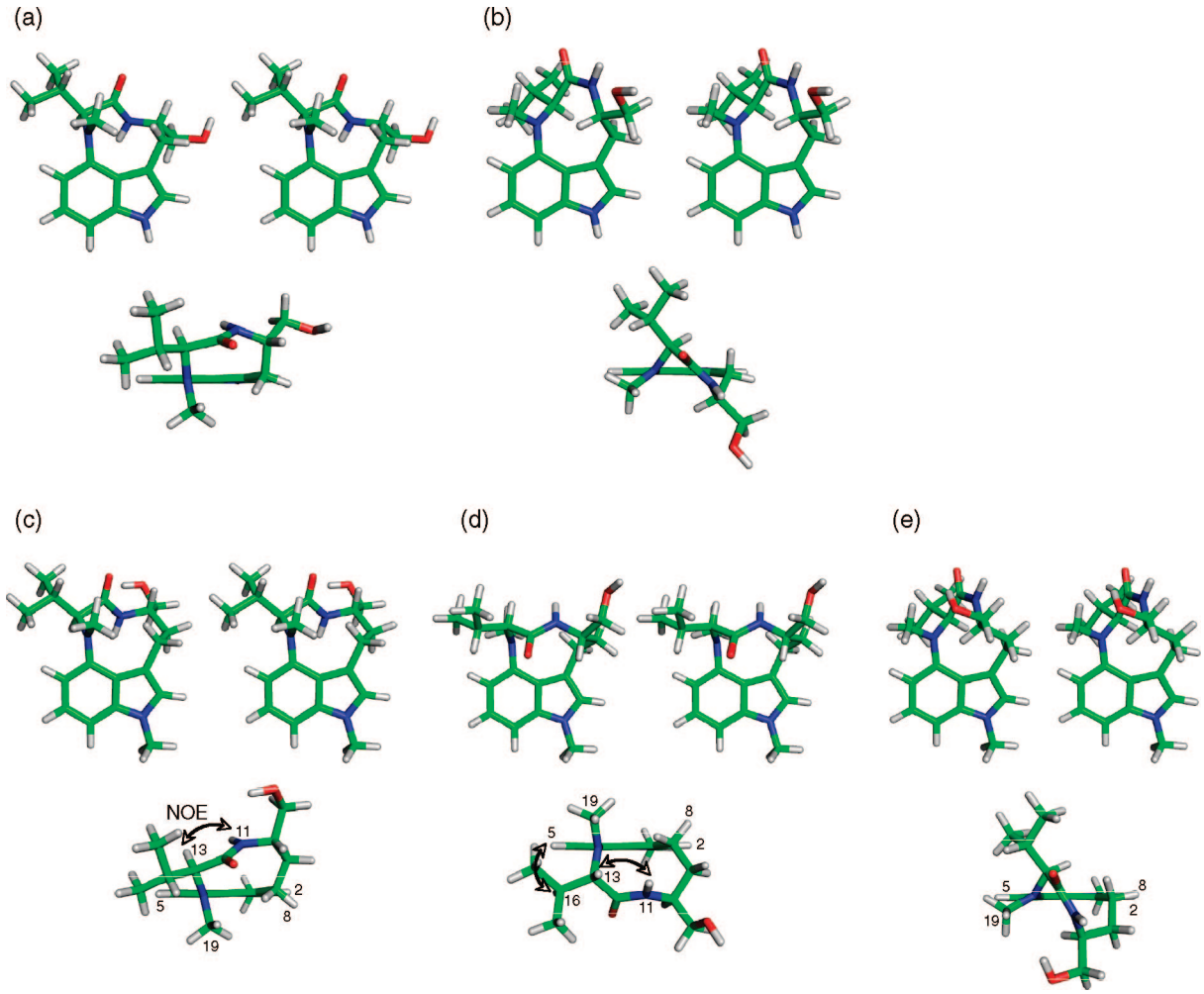


Figure 3. Stereoviews from the front and top views of the conformations of indolactam-V (**1**) and 1-hexylindolactam-V10 (**5**). The hexyl group at position 1 of **5** was displayed as a methyl group for convenience. Conformation of **1**: (a) the sofa form; (b) the twist form. Conformation of **5**: (c) conformer A, the sofalike form; (d) conformer B, the *r-trans*-fold-like form; (e) conformer C, the twistlike form. These names were used based on Itai's nomenclature.[32]

Table 2. K_i values of Indolactam-V (**1**), Benzolactam-V8 (**3**), 1-Hexylindolactam-V (**4**), and 1-Hexylindolactam-V10 (**5**) for Wild-Type δ-C1B and γ-C1A along with Their Fluorine-Substituted Mutants at Position 4 of Pro-11

	K_i (nM)			K_i (nM)		
compd	δ-C1B	δ-C1B(P11dfP)[a]	ratio	γ-C1A	γ-C1A(P11dfP)[a]	ratio
1	11 (0.6)[b]	130[c] (10)	11.8	89 (3.9)	690 (73)	7.8
3	410[c] (15)	436[c] (42)	1.1	3100 (300)	2500 (110)	0.8
4	0.46 (0.07)	3.7 (0.5)	8.0	15 (3.5)	71 (27)	4.7
5	15 (1.3)	120 (11)	8.0	11000 (300)	14000 (500)	1.3

[a] K_d values for δ-C1B(P11dfP) and γ-C1A(P11dfP) were 3.5 and 5.1 nM, respectively. [b] Standard deviation from at least two separate experiments. [c] Cited from ref 25.

electron-withdrawing group to position 7 as reported for the synthesis of the twist-restricted analogues of indolactam-V.[34,49,50] Although all attempts were unsuccessful, 5-chloro or 2-bromo derivatives of **5** (**14** and **15**) were obtained as byproducts (Scheme 1). The ^{1}H NMR studies showed that both **14** and **15** existed as a mixture of the sofalike and *r-trans*-fold-like form (**14**, sofa/*r-trans*-fold = conformer A/B = 39.9:1, 0.012 M in $CDCl_3$; **15**, sofa/*r-trans*-fold = conformer A/B = 4.7:1, 0.055 M in $CDCl_3$). The disappearance of the twistlike conformer (conformer C) seemed to be caused by avoidance of the steric hindrance between the chlorine atom at position 5 and the methyl group at position 19 in **14** and would be due to steric hindrance between the bromine atom at position 2 and the hydrogen atoms at position 8 in **15** (Figure 3). Both compounds were subjected to a binding assay using PKC C1 peptides to reveal that they did not inhibit the specific binding of [^{3}H]PDBu even at 10 μM. Since the introduction of a fluorine atom at position 6 and a bromine atom at position 7 in the indole ring of **1** slightly increased the activity,[51,52] the electron-withdrawing effect would not significantly lower the activity of **5**. To clarify whether the chlorine and bromine atoms in each ligand interfere with the binding of the receptors, the docking simulation between **5**, **14**, or **15** with the *cis*-amide form and the PKCδ C1B domain was carried out using the program GOLD[53] (version 3.2), providing the putative binding modes in which the chlorine and bromine atoms did not clash with the receptor (Figure 4). Moreover, the docking simulation between the conformers with the *trans*-amide of **5** (the sofalike and *r-trans*-fold-like forms) and PKCδ C1B domain indicated that both *trans*-amide conformers of **5** could form only three hydrogen

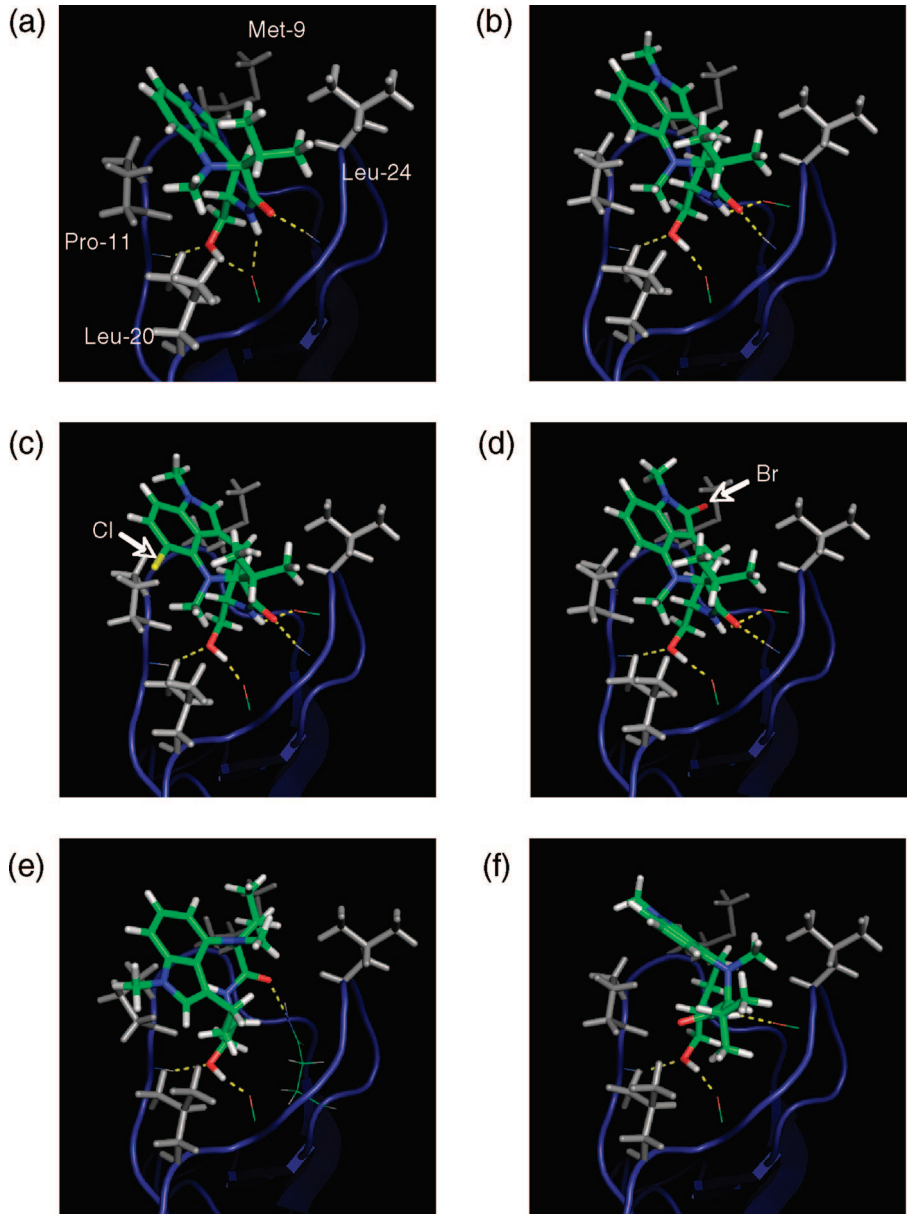


Figure 4. Docking simulation of indolactam-V (**1**), 1-hexylindolactam-V10 (**5**), 5-chloro-1-hexylindolactam-V10 (**14**), and 2-bromo-1-hexylindolactam-V10 (**15**) with the PKCδ C1B domain. The putative binding modes are as follows: (a) indolactam-V (**1**) (twist),[25] (b) **5** (twistlike), (c) **14** (twistlike), (d) **15** (twistlike), (e) **5** (sofalike), and (f) **5** (*r-trans*-fold-like). Hexyl groups of **5**, **14**, and **15** were replaced with methyl groups to simplify the calculation. Yellow dashed lines represent hydrogen bonds.

bonds with the receptor (parts e and f of Figure 4), whereas four hydrogen bonds were observed in the *cis*-amide form of **5**. These results suggest that the loss of the affinity of **14** and **15** could be due to the conformational restriction to *trans*-amide forms and that only the twistlike form of **5** could bind to the PKC C1 peptides.

In living cells, stimulation by PKC activators causes the translocation of PKC isozymes from the cytosol to the plasma membrane.[54] Recently, Garcia-Bermejo and colleagues reported that HK654, a conformationally restricted analogue of DAG without selectivity in either the binding or activation of PKCs, selectively translocated PKCα to the plasma membrane to induce apoptosis in LNCaP cells.[55] To verify whether the marked selectivity of **5** for nPKCs could also be observed in living cells, we carried out a PKC translocation assay in HeLa cells using green fluorescent protein (GFP) tagged PKC isozymes.[56,57] Each GFP-tagged PKC isozyme expressed in HeLa cells was stimulated by **5** at various concentrations. The translocation of GFP-tagged PKCδ to the plasma membrane is shown in Figure 5 as a typical example. The translocation data for all PKC isozymes are shown in Figure 6. To quantify the translocation, 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 intensity and the average cytosolic fluorescence intensity, respectively. The R values were plotted against time at various concentrations of **5**, and the maximum R values at each concentration are listed in Table 3. The time course of the translocation of GFP-tagged PKC isozymes induced by **5** along with **1** as a reference at 1 μM is also shown in Figure 7. Compound **5** selectively translocated nPKCs (δ, ϵ, η) over cPKCs at 0.1–1 μM. PKCβI slightly responded at 1 μM, but PKCα and PKCγ did not respond at all.

As another marker of the activation of PKCs, DsRed2-tagged myristoylated alanine-rich C kinase substrate (MARCKS) was coexpressed with GFP-tagged PKC isozymes. Under quiescent conditions, MARCKS was localized in the plasma membrane.

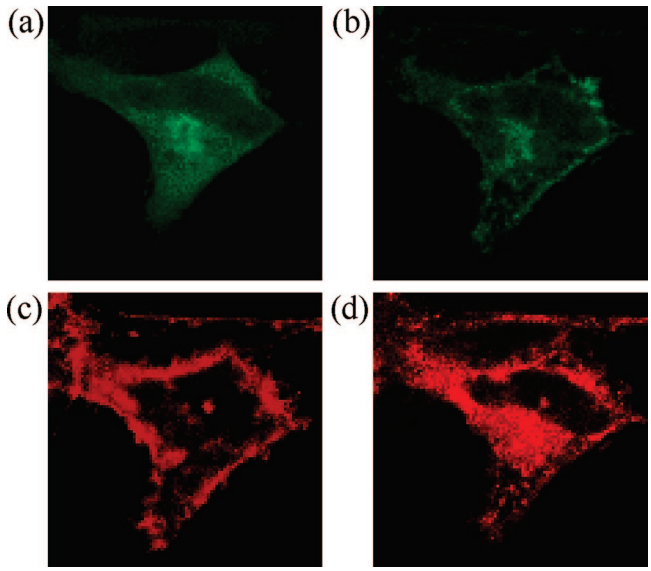


Figure 5. Maximal translocation of GFP-tagged PKCδ by 1-hexylindolactam-V10 (**5**). The fluorescence of GFP-tagged PKCδ in HeLa cells is as follows: (a) before and (b) after stimulation by **5** at 500 nM. The fluorescence of DsRed2-tagged MARCKS in HeLa cells is as follows: (c) before and (d) after stimulation by **5** at 500 nM.

However, MARCKS was translocated from the plasma membrane to the cytosol when phosphorylated by PKC.[58] After stimulation by **5**, translocation of DsRed2-tagged MARCKS could be monitored, confirming the activation of a PKC isozyme by **5**. Activation of PKCδ is shown in parts c and d of Figure 5 as a typical example. Similar results were obtained in PKCϵ and PKCη (data not shown). These results indicate that **5** could act as a selective activator of nPKCs in living cells.

Conclusion

In summary, we designed and synthesized 1-hexylindolactam-V10 (**5**), the ring expansion analogue of 1-hexylindolactam-V (**4**), as a potent PKC activator with selectivity for nPKCs that play an important role in many cellular processes including tumor promotion in mouse skin. Compound **5** exhibited marked selectivity for nPKCs over cPKCs, and its affinity for nPKC C1B domains was comparable to that of indolactam-V (**1**). Since **5** selectively translocated nPKCs over cPKCs in HeLa cells in the submicromolar range (0.1–1 μM), it would be useful for analyzing the mechanism of biological events related to the activation of nPKC. It would also be interesting to know how the nPKC-selective ligand **5** influences various tumor cell lines. As described above, recent investigations have revealed that a significantly different pattern of responses was triggered by PKCδ and PKCϵ in various cell lines and mouse epidermis.[7–11] Further efforts should be directed toward developing agents that distinguish between these nPKCs.

Experimental Section

General Remarks. The following spectroscopic and analytical instruments were used: UV, Shimadzu UV-2200A; digital polarimeter, Jasco DIP-1000; ^{1}H and ^{13}C NMR, Bruker ARX500, AVANCE400 (ref TMS); HPLC, Waters model 600E with model 2487 UV detector; (HR) EI-MS and FAB-MS, JEOL JMS-600H. HPLC was carried out on a YMC-packed SH-342-5 (ODS, 20 mm id × 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 (16.3 Ci/mmol) was purchased from PerkinElmer Life Sciences Research Products. All other chemicals and reagents were purchased from chemical companies and used without further purification.

Synthesis of 1-Hexylindolactam-V10 (5). NaH in oil (1.28 g, 32.0 mmol) was washed with hexane and suspended in dry DMF (20 mL) under an Ar atmosphere. To this suspension was added 4-nitroindole (4.07 g, 25.1 mmol) in dry DMF (30 mL) at 0 °C. After the mixture was stirred for 10 min, 1-iodohexane (4.4 mL, 29.9 mmol) was added dropwise and the reaction mixture was stirred for 2 h at 0 °C. The mixture was poured into H_2O and extracted with EtOAc. The 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 1-hexyl derivative (6.23 g).

To a solution of the 1-hexyl derivative (6.23 g) in pyridine (25.3 mL) was added dropwise pyridinium hydrobromide perbromide (8.50 g, 26.5 mmol) in pyridine (41 mL) at 0 °C. After being stirred for 30 min at 0 °C, the reaction mixture was poured into Et_2O and the precipitate was removed by filtration. The filtrate was washed successively with 1 M aqueous hydrochloric acid, 1 M aqueous sodium hydroxide, water, and brine and then 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 **7** (8.09 g, 24.9 mmol, 99%). Compound **7**: ^{1}H NMR δ (400 MHz, 296 K, $CDCl_3$, 0.031 M): 0.87 (3H, t, J = 7.0 Hz, –N(CH$_2$)$_5$CH_3), 1.24–1.34 (6H, m, –N(CH$_2$)$_2$(CH_2)$_3$CH$_3$), 1.82 (2H, m, –NCH$_2$CH_2–), 4.15 (2H, t, J = 7.2 Hz, –NCH_2–), 7.26 (1H, s, ArH-2), 7.28 (1H, t, J = 7.8 Hz, ArH-6), 7.59 (1H, dd, J = 7.8, 0.6 Hz, ArH-7), 7.74 (1H, dd, J = 7.8, 0.6 Hz, ArH-5). HR-FAB-MS m/z: 325.0550 (MH$^+$, calcd for $C_{14}H_{18}N_2O_2Br$, 325.0551).

A mixture of **7** (1.07 g, 3.30 mmol), **8** (1.76 g, 6.73 mmol), palladium(II) acetate (74.3 mg, 0.33 mmol), tri-*n*-butylphosphine (166 μL, 0.66 mmol), and K_2CO_3 (592 mg, 4.29 mmol) in DMF (15 mL) was stirred for 24 h at 80 °C under an Ar atmosphere and then filtered. To the filtrate was added water, and the mixture was extracted with EtOAc. The 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** (1.13 g, 2.23 mmol, 68%). Compound **9**: [α]$_D$ +51.4° (c 0.34, MeOH, 29.3°C). ^{1}H NMR δ (400 MHz, 296 K, $CDCl_3$, 0.028 M): 0.88 (3H, t, J = 7.0 Hz, –N(CH$_2$)$_5$CH_3), 1.24–1.31 (6H, m, –N(CH$_2$)$_2$(CH_2)$_3$CH$_3$), 1.57 (3H, s, –C(CH_3)$_2$), 1.70 (3H, s, –C(CH_3)$_2$), 1.84 (2H, t, J = 7.0 Hz, –NCH$_2$CH_2–), 3.90 (1H, m, –CH_2O–), 4.14 (2H, t, J = 7.2 Hz, –NCH_2–), 4.15 (1H, m, –CH_2O–), 4.58 (1H, m, –ArCH=CHCH–), 5.14 (2H, m, PhCH_2OC(O)–), 5.80 (1H, dd, J = 15.6, 7.8 Hz, ArCH=CH–), 6.87 (1H, d, J = 15.6 Hz, ArCH=CH–), 7.18–7.35 (7H, m, ArCH$_2$OC(O)–, ArH-2, and ArH-6), 7.59 (1H, d, J = 7.8 Hz, ArH-7), 7.85 (1H, d, J = 7.8 Hz, ArH-5). HR-FAB-MS m/z: 506.2677 (MH$^+$, calcd for $C_{29}H_{36}N_3O_5$, 506.2655).

To a solution of **9** (1.13 g, 2.23 mmol) in CH_2Cl_2 (22 mL) was added triethylamine (0.916 mL, 6.60 mmol) and 2,4,6-triisopropylbenzenesulfonyl hydrazide (3.93 g, 13.1 mmol), and the mixture was stirred for 18 h at room temperature under an Ar atmosphere. The reaction mixture was poured into water and was extracted with EtOAc. The 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 the saturated product (837 mg, 1.65 mmol, 74%).

To a solution of the saturated product (837 mg, 1.65 mmol) in MeOH (40 mL) was added *p*-toluenesulfonic acid monohydrate (323 mg, 1.70 mmol). After the mixture was stirred for 3 h at room temperature, the solvent was evacuated. The residue was purified by column chromatography on Wakogel C-200 using hexane and increasing amounts of EtOAc to give **10** (742 mg, 1.59 mmol, 96%). Compound **10**: [α]$_D$ –64.3° (c 0.10, MeOH, 29.3°C). ^{1}H NMR δ (400 MHz, 297 K, $CDCl_3$, 0.015 M): 0.87 (3H, t, J = 6.9 Hz, –N(CH$_2$)$_5$CH_3), 1.26–1.30 (6H, m, –N(CH$_2$)$_2$(CH_2)$_3$CH$_3$), 1.70 (2H, m, –NCH$_2$CH_2–), 1.76 (2H, m, ArCH$_2$CH_2–), 2.30 (1H, br s, OH),

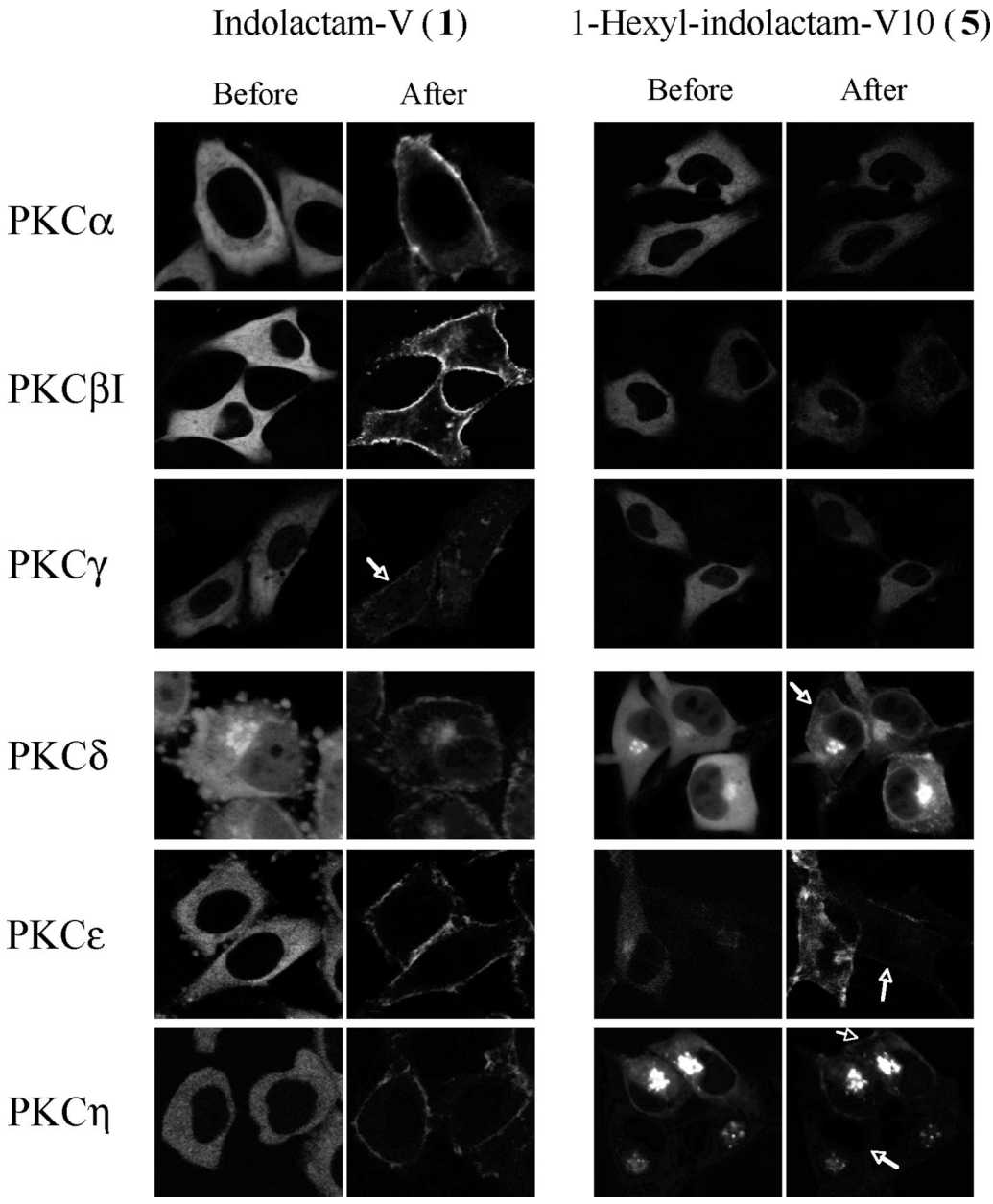


Figure 6. Maximal translocation of GFP-tagged PKC isozymes in HeLa cells induced by indolactam-V (**1**) and 1-hexylindolactam-V10 (**5**) at 1 μM. Arrows indicate the plasma membrane translocation. Each photograph shows the translocation at the time when each PKC isozyme was localized maximally to the plasma membrane.

Table 3. Maximal Translocation of GFP-Tagged PKC Isozymes Induced by Various Concentrations of 1-Hexylindolactam-V10 (**5**) and Indolactam-V (**1**) Expressed by the *R* Values[a]

	1-hexylindolacam-V10 (**5**)				
PKC isozymes	10 μM	1 μM	500 nM	100 nM	indolactam-V (**1**)1 μM
PKCα	1.77 (0.07)[b]	0.97 (0.01)	NT[c]	NT[c]	2.86 (0.22)
PKCβI	NT[c]	1.40 (0.04)	1.18 (0.01)	1.03 (0.04)	3.71 (0.58)
PKCγ	1.60 (0.2)	0.99 (0.02)	NT[c]	NT[c]	1.98 (0.23)
PKCδ	NT[c]	2.19 (0.19)	1.65 (0.03)	1.58 (0.24)	1.72 (0.05)
PKCϵ	NT[c]	2.95 (0.29)	2.50 (0.45)	2.47 (0.11)	3.98 (0.33)
PKCη	NT[c]	2.15 (0.20)	1.84 (0.16)	1.75 (0.10)	2.64 (0.29)

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

2.85 (1H, ddd, $J = 15.4, 9.6, 6.5$ Hz, ArCH_2–), 2.98 (1H, ddd, $J = 15.0, 9.7, 5.7$ Hz, ArCH_2–), 3.68–3.78 (3H, m, –C*H*(*N*HCbz)CH_2OH and –CH_2OH), 4.10 (2H, t, $J = 7.0$ Hz, –*N*CH_2–), 5.12 (3H, m, ArCH_2OC(O)–, –N*H*Cbz), 7.16 (1H, s, ArH-2), 7.21 (1H, t, $J = 8.0$ Hz, ArH-6), 7.29–7.42 (5H, m, *Ar*CH_2OC(O)–), 7.57 (1H, dd, $J = 8.0, 0.6$ Hz, ArH-7), 7.82 (1H, dd, $J = 8.0, 0.8$ Hz, ArH-5). HR-FAB-MS *m*/*z*: 468.2521 (MH^+, calcd for $C_{26}H_{34}N_3O_5$, 468.2498).

A mixture of **10** (742 mg, 1.59 mmol) and 10% Pd−C (148 mg) in MeOH (8.3 mL) was stirred vigorously under 1 atm of H_2 at room temperature for 1 h. The reaction mixture was filtered and then concentrated to give a crude amino alcohol. To a solution of

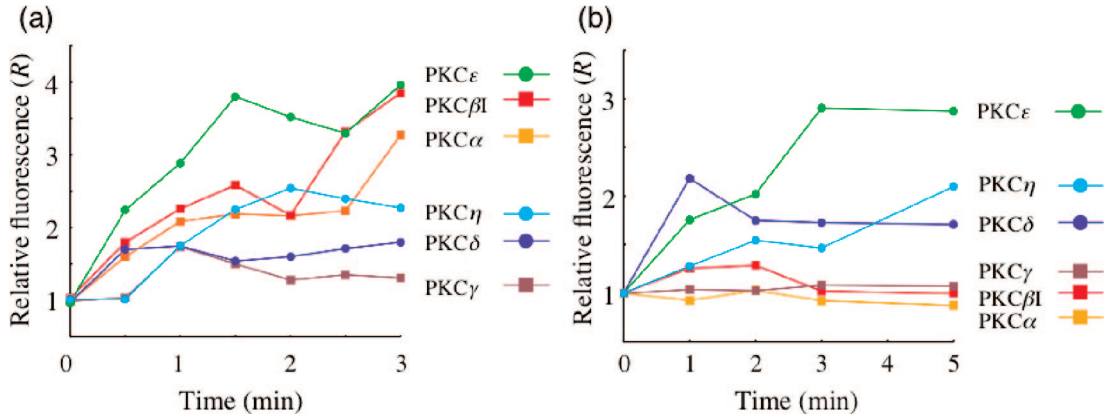


Figure 7. Time course of the translocation of GFP-tagged PKC isozymes to the plasma membrane induced by (a) indolactam-V (**1**) and (b) 1-hexylindolactam-V10 (**5**) at 1 μM. While the experiment was carried out several times, the results from one experiment are shown as representative. Similar results were obtained from other experiments.

the amino alcoholin H_2O (1.0 mL) and triethylamine (0.342 mL, 2.47 mmol) was added Boc-ON (438 mg, 1.78 mmol) in dioxane (0.98 mL) at 0 °C. After being stirred for 1 h at room temperature, the reaction mixture was poured into water and extracted with EtOAc. The 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 **11** (564 mg, 1.40 mmol, 88% in two steps). Compound **11**: $[\alpha]_D$ +51.8° (*c* 0.33, MeOH, 29.3°C). ^{1}H NMR δ (400 MHz, 298 K, $CDCl_3$, 0.015 M): 0.87 (3H, t, J = 7.0 Hz, –*N*$(CH_2)_5$C*H*$_3$), 1.24–1.32 (6H, m, –*N*$(CH_2)_2$(CH_2)$_3$$CH_3$), 1.45 (9H, s, –*O*C($CH_3$)$_3$), 1.77 (2H, m, –*N*$CH_2$C*H*$_2$–), 1.73–1.85 (1H, m, Ar$CH_2$C*H*$_2$–), 1.93–1.97 (1H, m, Ar$CH_2$C*H*$_2$–), 2.58 (1H, br.s, OH), 2.92 (2H, m, ArC*H*$_2$–), 3.63–3.72 (3H, m, –C*H*$_2$OH and –C*H*(NHBoc)CH_2OH), 3.96 (2H, t, J = 7.2 Hz, –*N*C*H*$_2$–), 4.12 (2H, br.s, ArN*H*$_2$), 4.84 (1H, br d, –N*H*Boc), 6.30 (1H, dd, J = 7.4, 0.6 Hz, ArH-7), 6.73 (1H, s, ArH-2), 6.76 (1H, dd, J = 8.3, 0.6 Hz, ArH-5), 6.97 (1H, dd, J = 8.3, 7.4 Hz, ArH-6). HR-EI-MS *m*/*z*: 403.2828 (M^+, calcd for $C_{23}H_{37}N_3O_3$, 403.2835).

To a solution of **11** (564 mg, 1.40 mmol) in THF (1.4 mL) was added acetic formic anhydride (1.03 mL) at 0 °C, which had been prepared by stirring a mixture of formic acid (0.39 mL) and acetic anhydride (0.64 mL) for 2 h at 50 °C. The mixture was stirred at 0 °C for 30 min and 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 then concentrated. The residue was purified by column chromatography on Wakogel C-200 using hexane and increasing amounts of EtOAc to give an amide. To a solution of the amide in THF (6.25 mL) was added dropwise 1.0 M BH_3 in THF solution (3.63 mL) at 0 °C, and the reaction mixture was stirred for 2 h at 0 °C. The reaction was quenched by addition of 50% THF/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 **12** (383 mg, 0.92 mmol, 66% in two steps). Compound **12**: $[\alpha]_D$ +300° (*c* 0.33, MeOH, 30.5°C). ^{1}H NMR δ (400 MHz, 298 K, $CDCl_3$, 9.9 mM): 0.87 (3H, t, J = 7.0 Hz, –*N*$(CH_2)_5$C*H*$_3$), 1.28–1.32 (6H, m, –*N*$(CH_2)_2$(CH_2)$_3$$CH_3$), 1.46 (9H, s, –OC(C*H*$_3$)$_3$), 1.75–1.84 (3H, m, –*N*CH_2C*H*$_2$– and ArCH_2C*H*$_2$–), 1.92–1,95 (1H, m, ArCH_2C*H*$_2$–), 2.85–2.98 (2H, m, ArC*H*$_2$–), 2.93 (3H, s, ArNHC*H*$_3$), 3.64–3.74 (3H, m, –C*H*$_2$OH and –C*H*(NHBoc)CH_2OH), 3.96 (2H, t, J = 7.1 Hz, –*N*C*H*$_2$–), 4.82 (1H, br s, ArN*H*CH_3), 6.22 (1H, d, J = 7.4 Hz, ArH-7), 6.69 (1H, s, ArH-2), 6.70 (1H, d, J = 8.5 Hz, ArH-5), 7.07 (1H, dd, J = 8.5, 7.4 Hz, ArH-6). HR-EI-MS *m*/*z*: 417.2997 (M^+, calcd for $C_{24}H_{39}N_3O_3$, 417.2991).

A mixture of **12** (383 mg, 0.92 mmol), 2,6-lutidine (0.31 mL, 2.76 mmol), and D-valine-derived triflate[20] (626 mg, 1.84 mmol) in 1,2-dichloroethane (3.0 mL) was refluxed at 70 °C for 22 h and then concentrated. The residue was purified by column chromatography on Wakogel C-200 using hexane and increasing amounts of EtOAc to give **13** (244 mg, 0.40 mmol, 44%). Compound **13**: $[\alpha]_D$ +26.3° (*c* 0.37, MeOH, 30.5°C). ^{1}H NMR δ (400 MHz, 298 K, $CDCl_3$, 9.9 mM): 0.88 (3H, t, J = 7.0 Hz, –*N*$(CH_2)_2$(CH_2)$_3$$CH_3$), 0.94 (3H, d, J = 6.6 Hz, –CH(C*H*$_3$)$_2$), 1.08 (3H, d, J = 6.8 Hz, –CH(C*H*$_3$)$_2$), 1.24–1.38 (6H, m, –*N*$(CH_2)_2$(CH_2)$_3$$CH_3$), 1.45 (9H, s, –*O*C(C*H*$_3$)$_3$), 1.73–1.83 (3H, m, –*N*CH_2C*H*$_2$– and ArCH_2C*H*$_2$–), 1.95 (1H, m, ArCH_2C*H*$_2$–), 2.31 (1H, m, –C*H*$(CH_3)_2$), 2.62 (1H, br s, –CH_2O*H*), 2.83 (3H, s, ArNC*H*$_3$), 2.89 (1H, m, ArC*H*$_2$–), 3.18 (1H, ddd, J = 14.4, 9.2, 5.1 Hz, ArC*H*$_2$–), 3.50 (1H, m, –C*H*$_2$OH), 3.60–3.66 (2H, m, –C*H*$_2$OH and –C*H*(NHBoc)CH_2OH), 3.75 (1H, d, J = 7.4 Hz, –*N*C*H*(CH(C*H*$_3$)$_2$)CO_2Bn), 4.01 (2H, t, J = 7.2 Hz, –*N*C*H*$_2$–), 4.75 (1H, br d, J = 6.8 Hz, –N*H*Boc), 4.96 (2H, s, –CO_2C*H*$_2$Ar), 6.82 (1H, dd, J = 7.2, 1.1 Hz, ArH-7), 6.83 (1H, s, ArH-2), 7.01 (1H, dd, J = 8.1, 7.2 Hz, ArH-6), 7.05 (1H, dd, J = 8.1, 1.1 Hz, ArH-5), 7.26–7.28 (5H, m, –CO_2CH_2*Ar*). HR-EI-MS *m*/*z*: 607.3994 (M^+, calcd for $C_{36}H_{53}N_3O_5$, 607.3985).

A mixture of **13** (238 mg, 0.392 mmol) and 10% Pd−C (23.8 mg) in MeOH (1.0 mL) was stirred vigorously under 1 atm of H_2 at room temperature for 1 h. The reaction mixture was filtered and then concentrated to give the monocarboxylic acid. To a solution of the monocarboxylic acid and *N*-hydroxysuccinimide (93.0 mg, 0.809 mmol) in CH_3CN (2.0 mL) was added a solution of DCC (119 mg, 0.623 mmol) in CH_3CN (1.5 mL) at 0 °C with stirring. The stirring was continued for 1 h at 0 °C and 2 h at room temperature, and then the solvent was removed in vacuo. The residue was suspended in EtOAc, and the insoluble urea was filtered off. The filtrate was concentrated and purified by column chromatography on Wakogel C-200 using hexane and increasing amounts of EtOAc to give the activated ester.

To a solution of the activated ester in CH_2Cl_2 (2.5 mL) was added TFA (2.5 mL) at 0 °C. The mixture was stirred for 1 h at room temperature, and then the solvent was removed in vacuo at below 30 °C. The residue was dissolved in EtOAc (25 mL), followed by the addition of saturated aqueous $NaHCO_3$ (3.0 mL). The mixture was refluxed for 1 h with vigorous stirring. The reaction mixture was poured into water and extracted with EtOAc. The 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, followed by HPLC on YMC SH-342-5 using 80% MeOH to give **5** (44.3 mg, 0.111 mmol, 28% in four steps). The purity of **5** was more than 98%, which was confirmed with two diverse HPLC systems on SH-342-5 using 80% MeOH (flow rate of 8.0 mL/min, retention time of 32.2 min) and 70% CH_3CN (flow rate of 8.0 mL/min, retention time of 18.2 min). Compound **5**: $[\alpha]_D$ +73.2° (*c* 0.92, MeOH, 29.9°C). UV λ_{max}(MeOH) nm (ϵ) 307 (6100), 230 (24 400). ^{1}H NMR δ (500 MHz, 300 K, $CDCl_3$, 0.050 M, sofa/*r*-*trans*-fold/twist = 7.2:1.4:1.0): for the sofalike form, 0.88 (3H, t, J = 7.0 Hz, H-25), 0.94 (3H, d, J = 6.5 Hz, H-18), 1.27 (3H, d, J = 6.7 Hz, H-17), 1.29 (6H, m, H-22–24), 1.40 (1H, m, H-9a), 1.78

(2H, m, H-21), 2.44 (2H, m, H-9b and H-16), 2.71 (1H, ddd, J = 15.1, 8.7, 2.0 Hz, H-8a), 2.85 (3H, s, H-19), 2.93 (1H, m, H-8b), 3.10 (1H, d, J = 10.8 Hz, H-13), 3.28 (1H, m, H-15a), 3.49 (1H, m, H-15b), 4.03 (2H, m, H-20), 4.30 (1H, m, H-10), 5.27 (1H, d, J = 9.6 Hz, H-13), 6.90 (1H, s, H-2), 6.97 (1H, dd, J = 7.0, 1.2 Hz, H-5), 7.11 (1H, dd, J = 8.2, 7.0 Hz, H-6), 7.14 (1H, dd, J = 8.2, 1.2 Hz, H-7); for the *r-trans*-fold-like form, 0.88 (3H, d, J = 6.4 Hz, H-18), 1.37 (3H, d, J = 5.3 Hz, H-17), 2.06 (1H, m, H-16), 3.06 (1H, m, H-10), 3.19 (3H, s, H-19), 3.23 (1H, d, J = 10.7 Hz, H-13), 5.53 (1H, d, J = 7.0 Hz, H-11), 6.94 (1H, s, H-2), 7.08 (1H, dd, J = 7.5, 1.3 Hz, H-5), 7.20 (1H, dd, J = 7.8, 1.3 Hz, H-7); for the twistlike form, 0.63 (3H, d, J = 6.8 Hz, H-18), 1.00 (3H, d, J = 6.3 Hz, H-17), 2.50 (1H, m, H-16), 2.94 (3H, s, H-19), 4.69 (1H, d, J = 9.1 Hz, H-13), 4.85 (1H, m, H-10), 6.48 (1H, d, J = 7.6 Hz, H-5), 6.88 (1H, d, J = 8.0 Hz, H-7), 6.91 (1H, s, H-2). Other peaks in the *r-trans*-fold-like and twistlike forms had weak intensities and overlapped those of the sofalike form. ^{13}C NMR δ (125 MHz, 300 K, $CDCl_3$, 0.112 M): for the sofa form, 14.01 (C-25), 19.97 (C-18), 20.12 (C-8), 20.17 (C-17), 22.54 (C-24), 25.45 (C-16), 26.71 (C-23), 20.03 (C-21), 31.41 (C-22), 35.59 (C-19), 35.69 (C-9), 46.47 (C-20), 50.17 (C-10), 65.85 (C-15), 75.32 (C-13), 107.26 (C-6), 117.27 (C-3), 120.61 (C-7), 121.89 (C-5), 124.52 (C-4′), 127.57 (C-2), 138.18 (C-7′), 146.35 (C-4), 173.27 (C-12). HR-EI-MS *m/z*: 399.2885 (M^+, calcd for $C_{24}H_{37}N_3O_2$, 399.2886).

Synthesis of 5-Chloro-1-hexylindolactam-V10 (14). To a solution of **5** (17.4 mg, 0.0436 mmol) in CH_2Cl_2 (1.0 mL) was added *N*-chlorosuccinimide (5.8 mg, 0.0434 mmol) at 0 °C, and the mixture was stirred for 15 min at 0 °C. The solvent was removed in vacuo, and the residue was purified by HPLC using 85% MeOH to give **14** (8.2 mg, 0.0189 mmol, 43%). Compound **14**: $[\alpha]_D$ −103° (*c* 0.34, MeOH, 24.7 °C). UV λ_{max} (MeOH) nm (ϵ): 316 (6640), 239 (28 200). ^{1}H NMR δ (400 MHz, 297 K, $CDCl_3$, 0.012 M, sofa/*r-trans*-fold = 39.9:1): for the sofalike-form, 0.88 (3H, t, J = 6.8 Hz, H-25), 0.98 (3H, d, J = 6.4 Hz, H-18), 1.22 (1H, dd, J = 11.2, 6.7 Hz, OH), 1.30 (6H, m, H-22–24), 1.36 (3H, d, J = 6.9 Hz, H-17), 1.42 (1H, m, H-9a), 1.77 (2H, m, H-21), 2.58 (2H, m, H-9b and H-16), 2.80 (2H, m, H-8), 2.92 (3H, s, H-19), 3.06 (1H, m, H-15a), 3.15 (1H, d, J = 10.5 Hz, H-13), 3.39 (1H, m, H-15b), 4.01 (2H, t, J = 7.2 Hz, H-20), 4.13 (1H, m, H-10), 5.32 (1H, br d, J = 7.8 Hz, H-10), 6.93 (1H, s, H-2), 7.08 (1H, d, J = 8.7 Hz, H-7), 7.21 (1H, d, J = 8.7 Hz, H-6); for the *r-trans*-fold-like form, 0.78 (3H, s, H-18), 3.22 (3H, s, H-19), 3.30 (1H, d, J = 9.1 Hz, H-13). Other peaks in the *r-trans*-fold-like had weak intensities and overlapped those of the sofalike form. ^{13}C NMR δ ($CDCl_3$, 0.038 M, 500 MHz, 300 K): for the sofalike form, 13.99 (C-25), 20.28 (C-8), 20.62 (C-17), 20.88 (C-18), 22.53 (C-24), 26.64 (C-23), 27.37 (C-16), 30.10 (C-21), 31.37 (C-22), 33.74 (C-9), 35.70 (C-19), 46.53 (C-20), 49.35 (C-10), 66.03 (C-15), 70.92 (C-13), 108.62 (C-7), 116.92 (C-3), 123.59 (C-6), 126.48 (C-5), 126.68 (C-4′), 127.92 (C-2), 136.86 (C-7′), 140.31 (C-4), 172.79 (C-12). HR-EI-MS *m/z*: 433.2493 (M^+, calcd for $C_{24}H_{36}N_3O_2Cl$, 433.2496).

Synthesis of 2-Bromo-1-hexylindolactam-V10 (15). To a solution of **5** (23.9 mg, 0.0599 mmol) in CH_2Cl_2 (1.0 mL) was added *N*-bromosuccinimide (11.7 mg, 0.0657 mmol) at 0 °C, and the mixture was stirred for 15 min at 0 °C. The solvent was removed in vacuo, and the residue was purified by HPLC using 85% MeOH to give **15** (13.1 mg, 0.0273 mmol, 46%). Compound **15**: $[\alpha]_D$ −98.2° (*c* 0.59, MeOH, 26.7 °C). UV λ_{max} (MeOH) nm (ϵ): 297 (8400), 229 (30 300). ^{1}H NMR δ (500 MHz, 300 K, $CDCl_3$, 0.055 M, sofa/*r-trans*-fold = 4.7:1): for the sofalike form, 0.88 (3H, t, J = 6.8 Hz, H-25), 0.94 (3H, d, J = 6.5 Hz, H-18), 1.27 (1H, d, J = 6.7 Hz, H-17), 1.30–1.34 (6H, m, H-22–24), 1.42 (1H, m, H-9a), 1.73 (2H, m, H-21), 2.37 (1H, m, H-9b), 2.46 (1H, m, H-16), 2.73 (1H, ddd, J = 15.3, 7.7, 2.3 Hz, H-8a), 2.81 (3H, s, H-19), 2.91 (1H, ddd, J = 15.3, 9.2, 2.3 Hz, H-8b) 3.09 (1H, d, J = 10.8 Hz, H-13), 3.31 (1H, dd, J = 10.5, 5.7 Hz, H-15a), 3.54 (1H, br d, J = 10.8 Hz, H-15b), 4.15 (1H, t, J = 7.6 Hz, H-20), 4.34 (1H, m, H-10), 5.11 (1H, br d, J = 9.7 Hz, H-11), 6.98 (1H, dd, J = 6.3, 2.2 Hz, H-5), 7.12 (2H, m, H-6,7); for the *r-trans*-fold-like form, 0.88 (3H, t, J = 6.8 Hz, H-25), 0.89 (3H, d, J = 5.9 Hz, H-18), 1.32–1.34 (6H, m, H-22–24), 1.37 (3H, d, J = 6.4 Hz, H-17), 1.73 (2H, m, H-21), 2.13 (1H, m, H-16), 2.13 (1H, m, H-16), 2.79 (1H, m, H-8a), 3.05 (1H, m, H-10), 3.16 (3H, s, H-19), 3.23 (1H, d, J = 10.7 Hz, H-13), 3.37 (1H, m, H-8b), 3.54 (1H, m, H-15a), 3.61 (1H, m, H-15b), 4.13 (2H, t, J = 7.6 Hz, H-20), 5.43 (1H, br d, J = 6.9 Hz, H-11), 7.12 (2H, m, H-5,6), 7.18 (1H, d, J = 7.5 Hz, H-7). ^{13}C NMR δ ($CDCl_3$, 0.055 M, 500 MHz, 300 K): for the sofalike form, 14.00 (C-25), 19.93 (C-18), 20.23 (C-17), 20.37 (C-9), 22.54 (C-24), 25.40 (C-16), 26.53 (C-23), 29.68 (C-21), 31.46 (C-22), 32.50 (C-8), 35.67 (C-19), 45.38 (C-20), 50.44 (C-10), 65.67 (C-15), 75.40 (C-13), 107.35 (C-6), 115.12 (C-2), 116.05 (C-3), 121.43 (C-5), 122.29 (C-7), 124.06 (C-4′), 138.03 (C-7′), 145.40 (C-4), 172.85 (C-12). HR-EI-MS *m/z*: 477.1993 (M^+, calcd for $C_{24}H_{36}N_3O_2Br$, 477.1991).

Conformational Analysis of 1-Hexylindolactam-V10 (5). A conformational search was carried out using the random search function in Sybyl 7.1 (Tripos, Inc.). The hexyl group of **5** was replaced with a methyl group to simplify the calculation. The following conditions and parameters were used: all rotatable bonds were perturbed; the energy cutoff was set to 15 kcal/mol; the maximum number of search iterations and the maximum number of hits were both set to 100 000; 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^{-1} Å^{-1}. As a result, 62 conformations with energy values within 10 kcal/mol from the global minimum were obtained and classified into eight groups (sofa, *r-trans*-fold, *trans*-fold, *r*-sofa, *r-trans*-fold (two), fold, *cis*-sofa, and twist) on the basis of Itai's nomenclature (Supporting Information).[32]

Inhibition of Specific [^{3}H]PDBu Binding to PKC Isozyme C1 Peptides. The binding of [^{3}H]PDBu to the PKC C1 peptides was evaluated using the procedure of Sharkey and Blumberg[41] with modifications as reported previously[37] with 50 mM Tris-maleate buffer (pH 7.4 at 4 °C), 10–40 nM PKC C1 peptide, 20 nM [^{3}H]PDBu (16.3 Ci/mmol), 50 μg/mL 1,2-dioleoyl-*sn*-glycero-3-phospho-L-serine, 3 mg/mL bovine γ-globulin, and various concentrations of inhibitor. Binding affinity was evaluated on the basis of concentration required to cause 50% inhibition of the specific binding of [^{3}H]PDBu, IC_{50}, which was calculated with PriProbit 1.63 software.[59] The inhibition constant, K_i, was calculated using the method of Sharkey and Blumberg.[41] Although we used each PKC C1 peptide in the range 10–40 nM, the concentration of the properly folded peptide was estimated to be about 3 nM based on the B_{max} values of the Scatchard analyses reported previously.[37] Therefore, the concentration of free PDBu will not markedly vary over the dose response curve.

Synthesis of γ-C1A(P11dfP). γ-C1A(P11dfP) was prepared by solid-phase Fmoc synthesis as reported previously.[25] The K_d value of [^{3}H]PDBu for γ-C1A(P11dfP) in the presence of 1,2-dioleoyl-*sn*-glycero-3-phospho-L-serine was 5.1 nM. The identity and purity of the peptide were confirmed by MALDI-TOF-MS [average molecular mass, 6133.10 (MH^+, calcd 6132.61)] and HPLC (>98% pure), respectively.

Docking Study. The crystal structure of the PKCδ C1B domain[60] (PDB code 1ptq) was obtained from the Protein Data Bank. Hydrogen atoms were added to the protein, and all of the hydrogens were energetically minimized, keeping all heavy atoms fixed. The hexyl groups of the ligands were replaced with methyl groups to simplify the calculation. Each ligand was docked using GOLD[53] (version 3.2) in 50 independent genetic algorithm (GA) runs, and for each of these a maximum number of 100 000 GA operations were performed on a single population of 50 individuals. Operator weights for crossover, mutation, and migration in the entry box were used as default parameters (95, 95, and 10, respectively), as well as the hydrogen bonding (4.0 Å) and van der Waals (2.5 Å) parameters, and the ChemScore scoring function was selected. The active site was created around ϵ-NH of Gln-27, and the radius was set to 10 Å, with the automatic active-site detection on. The top-ranked solutions were geometrically optimized at the protein

side chains within 2 Å from the ligand and allowed to relax using SYBYL 7.3 with an MMFF94s force field.

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 medium was then replaced with 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. Translocation of the GFP-tagged PKCs was triggered by addition of **5** 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 fluorescence 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 determine the translocation to the plasma membrane quantitatively, time series of confocal fluorescence images of cells expressing GFP-tagged PKCs were recorded before and after stimulation with **5**. 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 **5** was added at various concentrations, the R values were plotted against time (Figure 7). The maximum R value represents the maximum membrane translocation of each PKC in response to **5** at various concentrations, and results are summarized in Table 3.

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Supporting Information Available: NMR spectra of **5** including data from a variable-temperature study and NOESY experiment. This material is available free of charge via the Internet at http://pubs.acs.org.

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