A Synthesis of 2-Substituted 2-Aminoethanol Derivatives Having Inhibitory Activity against Protein Kinase C

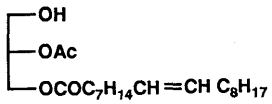
Tameo IWASAKI,* Hiroyoshi YAMAZAKI, Takashi NISHITANI, Kazuhiko KONDO, and Tadashi SATO*

Research Laboratory of Applied Biochemistry, Tanabe Seiyaku Co., Ltd., 3–16–89 Kashima, Yodogawa, Osaka 532, Japan. *Received July 15, 1991*

A series of 2-aminoethanol derivatives was synthesized and their inhibitory activities against protein kinase C were investigated. Among these compounds, 2-*endo*-hexadecylamino-5-norbornene-2-*exo*-methanol (4h) and 2-*endo*-hexadecylamino-5-norbornene-2,3-*exo*-dimethanol (4i) inhibited protein kinase C at the IC_{50} values of 2×10^{-5} and 1×10^{-5} M, respectively, but not protein kinase A at a concentration of 1×10^{-3} M. The structure–activity relationships are discussed.

Keywords protein kinase C; aminonorbornene; 2-aminoethanol; protein kinase C inhibitor; aminonorbornane

Protein kinase C, a key enzyme in signal transduction and cell regulation, is activated physiologically by diacylglycerol produced by the receptor-mediated turnover of inositol phospholipids.[1] This enzyme is also the principal target of phorbol esters[2] and other tumor promoters.[3] To elucidate the physiological role of protein kinase C and to develop agents that can selectively prevent the effect of tumor promoters, a number of natural and synthetic inhibitors have been investigated. These include microbial products, such as polymyxin B,[4] staurosporine,[5] K-252a,[6] and UCN-1028A,[7] and synthetic inhibitors, such as chlorpromazine,[8] quaternary ammonium derivatives of alkylglycerols,[9] and naphthalenesulfonamide derivative (H-7).[10] Of these inhibitors, UCN-1028A, isolated from the culture broth of *Cladosporium cladosporioides*, was recently reported to be a specific inhibitor of this enzyme.[7]



diacylglycerol
(1-oleoyl-2-acetylglycerol)

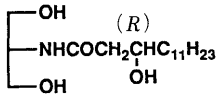


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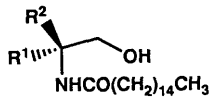
Chart 1

On the other hand, Hannun *et al.*[11] have reported that lisosphingolipids possessing an aminoethanol skeleton inhibit protein kinase C, and may have a physiological role as negative modulators of this enzyme.

On the basis of this information, we envisaged that the replacement of one of the oxygen atoms of the glycerol skeleton of diacylglycerol with a nitrogen atom would provide an inhibitor of protein kinase C. Thus, we synthesized a variety of 2-substituted *N*-palmitoyl- and *N*-hexadecyl-2-aminoethanols, and found that 2-*endo*-hexadecylamino-5-norbornene-2,3-*exo*-dimethanol (**4i**), a sterically defined 2-aminoethanol, exhibited the most potent activity against protein kinase C.[12] Herein, we wish to report the synthesis and structure–activity relationships of these compounds.

Chemistry Compound **1** was synthesized starting from L-serine benzyl ester (**8**). Acylation of **8** with *N*-((3*R*)-3-hydroxytetradecanoyl)succinimide (**9**) in the presence of Et_3N, followed by $NaBH_4$-reduction of the resulting ester (**10**) afforded **1** in a good overall yield. Similarly, the other 2-substituted 2-aminoethanols (**2a—d**) were synthesized in good yields by acylation of the corresponding α-substituted α-amino acid esters with palmitoyl chloride, followed by $LiAlH_4$-reduction of the resulting palmitoylamino acid

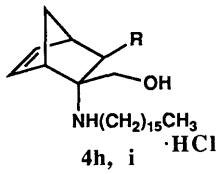


2a-d

a: R^1=H, R^2=H
b: R^1=H, R^2=Me
c: R^1=R^2=Me
d: R^1, R^2=-$(CH_2)_4$-

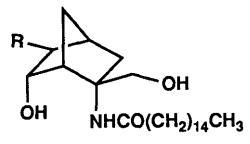
3e-g

e: R^1=H, R^2=H
f: R^1=H, R^2=CH_2OH
g: R^1=Ac, R^2=H



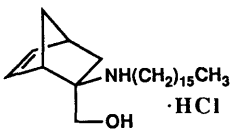
4h, i

h: R=H
i: R=CH_2OH



5j, k

j: R=I
k: R=H



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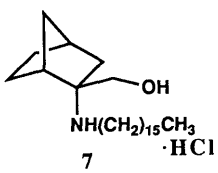

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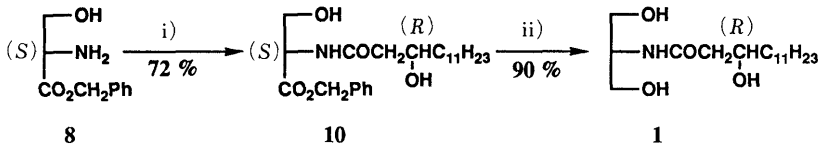
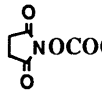
Chart 2

esters.

Synthesis of 2-aminoethanol derivatives having a norbornyl group is summarized in Chart 4. Compounds **3e** and **3f** were synthesized from 2-*endo*-acetamido-5-norbornene-2-*exo*-carboxylic acid methyl ester (**11m**)[13,14] and 2-*endo*-acetamido-5-norbornene-2,3-*exo*-dicarboxylic acid dimethyl ester (**11n**),[14] respectively, by a series of procedures involving deacetylation of **11m, n** with the Meerwein's reagent, acylation of **12m, n** with palmitoyl chloride and $LiAlH_4$-reduction of the resulting palmitoyl-

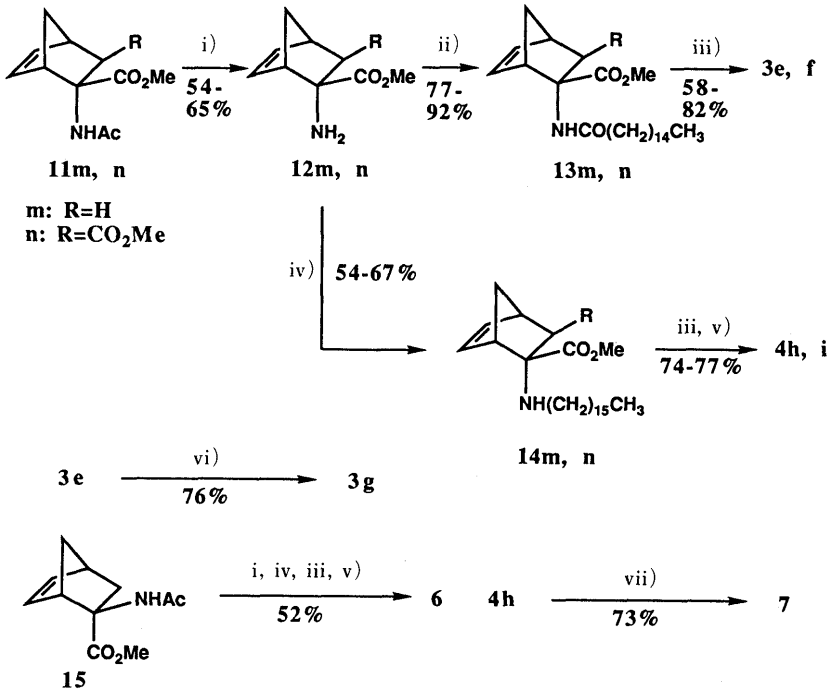


8 **10** **1**

i)  (R)
$NOCOCH_2CH(OH)C_{11}H_{23}$(9), Et_3N/THF, r.t.

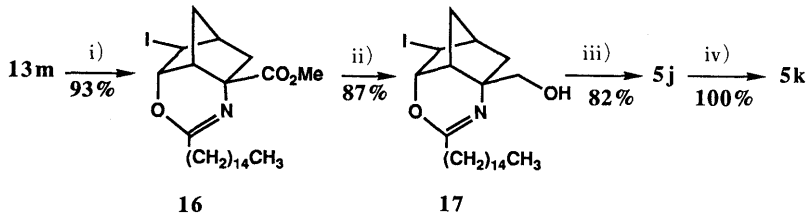
ii) $NaBH_4$/EtOH, r.t.

Chart 3



i) a: $Et_3O^+BF_4^-/CH_2Cl_2$, r.t., b: aqueous $NaHCO_3$; ii) $CH_3(CH_2)_{14}COCl$, Et_3N/THF, r.t.; iii) $LiAlH_4$/THF, 5°C–r.t.; iv) $CH_3(CH_2)_{15}Br$, K_2CO_3/ HMPA, r.t.–70°C; v) HCl/MeOH; vi) Ac_2O, Et_3N/THF; vii) H_2/Pd-C, MeOH

Chart 4



i) I_2/CH_2Cl_2, CH_3CN, r.t.; ii) $Ca(BH_4)_2$/EtOH, 5°C;
iii) 10% aqueous AcOH solution, 60-70°C; iv) H_2, Pd-C/MeOH, r.t.

Chart 5

amino acid esters (**13m, n**). Alkylated compounds, **4h** and **4i**, were also synthesized by alkylation of **12m** and **12n** with hexadecyl bromide in hexamethylphosphoramide (HMPA) in the presence of K_2CO_3, followed by $LiAlH_4$-reduction of **14m** and **14n**, respectively. *O*-Acetylated compound (**3g**) was synthesized by acetylation of **3e** with Ac_2O.

The stereoisomer (**6**) of **4h** was also synthesized from the stereoisomer (**15**)[14] of **11m**, by the same procedure as employed in the preparation of **4h**. Furthermore, the norbornane derivative (**7**) was synthesized by hydrogenation of **4h**.

The introduction of a hydroxyl group into the 6-*endo* position of the norbornyl group was achieved in a regio- and stereoselective manner. Thus, the reaction of the compound **13m** with I_2, followed by $Ca(BH_4)_2$-reduction of the ester (**16**) and subsequent acid catalyzed cleavage of the oxazoline ring of **17** afforded **5j** in a good overall yield. Reduction of **5j** thus obtained furnished **5k** in a quantitative yield.

Biological Results and Discussion

The inhibitory activity of the compounds synthesized above against protein kinase C prepared from rat brain cytosol was measured under the assay conditions reported by Kikkawa *et al.*[15] The results are summarized in Table I.

Compound **1**, in which one of the oxygen atoms of the glycerol skeleton of diacylglycerol is replaced by a nitrogen atom, exhibited the inhibitory activity against protein kinase C at the IC_{50} value of 2×10^{-4}. This indicates that the 2-aminoethanol skeleton is important for exhibiting the inhibitory activity. In order to examine the effect of the substituent on the 2-position of 2-aminoethanol skeleton, we investigated the inhibitory activity of (*S*)-2-methyl-2-(*N*-palmitoylamino)ethanol (**2b**) in which one of the hydroxymethyl groups of **1** is replaced by a methyl group, and found that this compound retained the inhibitory activity. We then investigated the significance of the bulkiness of the substituent at the 2-position of 2-aminoethanol skeleton by examining the inhibitory activity of a series of 2-substituted 2-aminoethanols (**2a—d**). The results are shown in Table I. These results suggest that the inhibitory activity of these compounds increased with increase of the bulkiness of the substituent on the 2-position. On the other hand, the norbornyl group is intriguing not only because of being more bulky than the cyclopentyl group, but also because it has a conformational rigidity which would result in a specific biological activity.[16] Thus, in order to find a compound having more potent activity, we synthesized and tested the norbornene derivatives (**3e, f, 4h, i**), and found that they inhibited protein kinase C at the IC_{50} values of the order of 10^{-5} M. The inhibitory activities of these compounds, showed that introduction of a hydroxymethyl group into the 3-position of the norbornene skeleton did not significantly alter the activity whereas replacement of the *N*-palmitoyl group with the *N*-hexadecyl group increased the activity. Furthermore, we examined the inhibitory activity of **7**. As a result, **7** showed almost the same activity as that of **4h**, indicating that the double bond of **4h** does not significantly contribute to the activity.

Next, we examined the effect of a hydrophilic group at the 6-position of the norbornane skeleton. Compound **5k** having a hydroxyl group at the 6-*endo*-position was found to exhibit a good level of inhibitory activity, indicating that a hydrophilic group at the 6-position does not affect the activity. However, in the case of the compound **5j**, which has the 5-*exo*-iodo group and the 6-*endo*-hydroxyl group, no inhibitory activity was observed, suggesting that the bulkiness of the 5-iodo group is too bulky to retain the activity.

In order to get an insight into both the effect of the stereochemistry of the 2-aminoethanol moiety and the significance of the 2-hydroxymethyl group of the norbornene skeleton, we investigated the inhibitory activities of the stereoisomer (**6**) of **4h** and the *O*-acetyl derivative (**3g**) of **3e**. The inhibitory activities of these compounds were significantly reduced, strongly indicating that the 2-*exo*-hydroxymethyl and 2-*endo*-amino groups are crucial for exhibiting the inhibitory activity. The results obtained above imply the significance of the norbornyl skeleton which is capable of sterically defining both the amino and hydroxymethyl groups.

Among the compounds described above, we selected **4h** and **4i** for further evaluation. We examined the inhibition of the binding of [^{3}H]phorbol dibutyl ester (PDBu) to protein kinase C by compounds (**4h, i**) *in vitro* under the assay conditions reported by Tanaka *et al.*[17] Both **4h** and **4i** were found to competitively inhibit the [^{3}H]PDBu binding to protein kinase C at the IC_{50} value of 2×10^{-5} M (Table II). No inhibitory activities against protein kinase A[15] were observed for **4h** and **4i** at a concentration of 1×10^{-3} M (Table II), nor did these compounds show any activation of protein kinase C.[18] These results indicate that **4h** and **4i** are specific inhibitors of protein kinase C.

TABLE I. Inhibitory Activities of 2-Substituted 2-Aminoethanol Derivatives[a] against Protein Kinase C

Compound	IC_{50} (M)	Compound	IC_{50} (M)
1	2×10^{-4}	**3g**	$>10^{-3}$
2a	$>10^{-3}$	**4h**	2×10^{-5}
2b	6×10^{-4}	**4i**	1×10^{-5}
2c	2×10^{-4}	**5j**	$>10^{-3}$
2d	8×10^{-5}	**5k**	6×10^{-5}
3e	5×10^{-5}	**6**	$>10^{-3}$
3f	5×10^{-5}	**7**	2×10^{-5}

a) The α-carbon on amino group of **2a** is (*S*)-configuration. The others are racemic compounds.

TABLE II. Inhibitory Activities of **4h** and **4i** against [^{3}H]PDBu Binding to Protein Kinase C and against Protein Kinase A

Compound	Binding of [^{3}H]PDBu to protein kinase C IC_{50} (M)	Protein kinase A IC_{50} (M)
4h	2×10^{-5}	$>10^{-3}$
4i	2×10^{-5}	$>10^{-3}$

Experimental

All melting points were uncorrected. Infrared (IR) spectra were recorded on a Shimadzu IR-420 infrared spectrophotometer. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were taken at 200 MHz on a Bruker AC-200 spectrometer with tetramethylsilane as an internal reference. Mass spectra (MS) were given by a Hitachi M-60 instrument.

***N*-((3*R*)-3-Hydroxytetradecanoyl)-L-serine Benzyl Ester (10)** Et_3N (3.9 ml, 28.0 mmol) was added to a solution of **8** (9.9 g, 28.0 mmol) and **9** (9.5 g, 28.0 mmol) in tetrahydrofuran (THF, 150 ml). The reaction mixture was stirred at room temperature for 20 h. The mixture was evaporated to dryness *in vacuo*. The residue was dissolved in $CHCl_3$ and the solution was washed with aqueous $NaHCO_3$ and H_2O. The organic layer was separated, dried ($MgSO_4$) and then evaporated to dryness *in vacuo* to afford **10** (9.0 g, 76%) as crystals. mp 120—121 °C. ^{1}H-NMR (DMSO-d_6) δ: 0.83 (t, 3H), 1.00—1.50 (m, 20H), 2.21 (d, 2H), 3.50—3.90 (m, 3H), 4.20—4.40 (m, 1H), 4.43 (t, 1H), 4.48 (d, 1H), 5.02 (s, 2H), 7.20 (s, 5H), 7.98 (d, 1H). $[\alpha]_D$ −10.9° (c=1, MeOH). *Anal.* Calcd for $C_{24}H_{39}NO_5$: C, 68.38; H, 9.32; N, 3.32. Found: C, 68.69; H, 9.47; N, 3.39.

2-((3*R*)-3-Hydroxytetradecanoyl)amino-1,3-propanediol (1) $NaBH_4$ (1.4 g, 38.0 mmol) was added to a solution of **10** (8.0 g, 19.0 mmol) in EtOH (250 ml). The reaction mixture was stirred at room temperature for 5 h, then neutralized with AcOH. The solution was evaporated to dryness *in vacuo*. The residue was triturated with H_2O and the resulting crystals were collected by filtration. Recrystallization from MeOH gave **1** (5.4 g, 90%). mp 121—123 °C. ^{1}H-NMR (DMSO-d_6) δ: 0.83 (t, 3H), 1.23 (m, 20H), 2.15 (d, 2H), 3.29—3.90 (m, 7H), 4.30—4.60 (m, 3H), 7.30 (d, 1H). $[\alpha]_D$ −6.7° (c=1, MeOH). *Anal.* Calcd for $C_{17}H_{35}NO_4$: C, 64.32; H, 11.11; N, 4.41. Found: C, 64.53; H, 11.40; N, 4.51.

Typical Procedure for the Acylation with Palmitoyl Chloride and Subsequent $LiAlH_4$-Reduction 2,2-Dimethyl-2-(*N*-palmitoylamino)ethanol (**2c**): Palmitoyl chloride (5.5 g, 20.0 mmol) was added to a solution of 2-aminoisobutyric acid methyl ester (2.3 g, 20.0 mmol) and Et_3N (5.6 ml, 40.0 mmol) in THF (30 ml) under ice cooling. The reaction mixture was stirred at room temperature for 20 h, then evaporated to dryness *in vacuo*. The residue was dissolved in $CHCl_3$ and the solution was washed with aqueous $NaHCO_3$ and H_2O. The organic layer was separated, dried ($MgSO_4$) and then evaporated to dryness *in vacuo* to afford the palmitoylamino ester (5.2 g, 73%). A solution of the above ester in THF (80 ml) was added to a suspension of $LiAlH_4$ (0.57 g, 15.0 mmol) in THF (20 ml) under ice cooling. After the reaction mixture was stirred for 45 min at room temperature, the reaction was quenched by addition of 15% aqueous NaOH. The insoluble materials were filtered off and the filtrate was evaporated to dryness *in vacuo*. The resulting crystals were triturated with hexane to afford **2c** (4.0 g, 82%) as colorless needles. mp 57—58 °C (hexane). IR (Nujol): 3500, 3180, 1625, 1550 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.61—2.30 (m, 37H), 3.53 (s, 2H), 4.45 (br, 1H), 5.73 (br, 1H). MS *m/z*: 328 (M^++1).

The other 2-(*N*-palmitoylamino)ethanol derivatives were prepared from the known amino acid esters[19] by the same procedure. The melting points of these compounds are as follows: **2a**, 94—96 °C (AcOEt); **2b**, 85—86 °C (AcOEt–hexane); **2d**, 76—77 °C (AcOEt–hexane).

2-*endo*-Amino-5-norbornene-2-*exo*-carboxylic Acid Methyl Ester (**12m**): Meerwein's reagent (2.29 g, 5.0 mmol) was added to a solution of **11m** (1.05 g, 5.0 mmol) in CH_2Cl_2 (20 ml) all at once at room temperature. The reaction mixture was stirred at room temperature for 2 h. Cold aqueous $NaHCO_3$ (10 ml) was added to the mixture and the reaction mixture was stirred for 18 h. The organic layer was separated, dried ($MgSO_4$) and then evaporated to dryness *in vacuo*. The residue was purified by column chromatography on silica gel ($CHCl_3$: EtOH = 30 : 1) to afford **12m** (0.55 g, 65.4%) as a faintly yellow syrup. ^{1}H-NMR ($CDCl_3$) δ: 0.80—1.10 (m, 1H), 1.20—1.80 (m, 2H), 1.59 (s, 2H), 2.45—2.70 (m, 1H), 2.80—3.10 (m, 2H), 3.78 (s, 3H), 6.10—6.30 and 6.40—6.55 (m and m, 2H). MS *m/z*: 167 (M^+).

2-*exo*-Hydroxymethyl-2-*endo*-palmitoylamino-5-norbornene (**3e**): Palmitoyl chloride (12.6 g, 45.8 mmol) was added to a solution of **12m** (7.7 g, 45.8 mmol), Et_3N (7.6 ml, 55.0 mmol) and dimethylaminopyridine (DMAP, 0.1 g) in THF (50 ml) under ice cooling. The reaction mixture was stirred at room temperature for 1 h, then diluted with AcOEt and the solution was washed with H_2O. The organic layer was separated, dried ($MgSO_4$) and evaporated to dryness *in vacuo*. The resulting syrup was purified by column chromatography on silica gel ($CHCl_3$: AcOEt = 2 : 1) to afford **13m** as crystals (17.1 g, 92%). A solution of **13m** (4.5 g, 10.0 mmol) in THF (15 ml) was added to a suspension of $LiAlH_4$ (0.38 g, 10.0 mmol) in THF (40 ml) under ice cooling. After the reaction mixture was stirred for 4 h at room temperature, the reaction was quenched by addition of 15% aqueous NaOH. The insoluble materials were filtered off and the filtrate was evaporated to dryness *in vacuo*. The resulting syrup was purified by column chromatography on silica gel ($CHCl_3$: Me_2CO = 10 : 1) to afford **3e** as crystals (3.1 g, 82%). mp 94—96 °C. IR (Nujol): 3190, 1630 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.75—1.00 (m, 3H), 1.00—1.80 (m, 29H), 1.90—2.20 (m, 3H), 2.85 (br, 1H), 3.32 (br, 1H), 3.80 (d, 2H), 5.03 (t, 1H), 5.40—5.55 (m, 1H), 5.90—6.05 and 6.20—6.35 (m and m, 2H). MS *m/z*: 377 (M^+). *Anal.* Calcd for $C_{24}H_{43}NO_2$: C, 76.34; H, 11.48; N, 3.71. Found: C, 76.51; H, 11.52; N, 3.68.

2-*endo*-Palmitoylamino-5-norbornene-2,3-*exo*-dimethanol (**3f**): This compound was prepared from **11n** by the same procedure as that described for the synthesis of **3e** (24% overall yield). mp 90—91 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.80—1.00 (m, 3H), 1.10—1.90 (m, 29H), 2.00—2.20 (m, 2H), 2.55 (br, 1H), 3.23 (br, 1H), 3.50—4.10 (m, 4H), 4.00—4.40 (br, 1H), 5.00—5.20 (m, 1H), 5.77 (s, 1H), 6.00—6.15 and 6.30—6.45 (m and m, 2H). MS *m/z*: 407 (M^+). *Anal.* Calcd for $C_{25}H_{45}NO_3$: C, 73.66; H, 11.13; N, 3.44. Found: C, 73.89; H, 11.35; N, 3.31.

2-*endo*-Hexadecylamino-5-norbornene-2-*exo*-methanol Hydrochloride (**4h**): Hexadecyl bromide (1.9 g, 6.0 mmol) and K_2CO_3 (1.03 g, 7.5 mmol) were added to a solution of **12m** (0.84 g, 5.0 mmol) in HMPA (1 ml). The reaction mixture was stirred at room temperature for 4 h, then diluted with AcOEt. The solution was washed with H_2O. The organic layer was separated, dried ($MgSO_4$) and then evaporated to dryness *in vacuo*. The resulting syrup was purified by column chromatography on silica gel (hexane : AcOEt = 4 : 1) to afford **14m** as a syrup (1.3 g, 67%). A solution of **14m** (1.3 g, 3.3 mmol) in THF (10 ml) was added to a suspension of $LiAlH_4$ (0.12 g, 3.3 mmol) in THF (10 ml) under ice cooling. After the reaction mixture was stirred at 20 °C for 45 min, the reaction was quenched by addition of 15% aqueous NaOH. The insoluble materials were filtered off and the filtrate was evaporated to dryness *in vacuo*. The resulting syrup was dissolved in 3 ml of 22% HCl in MeOH. The solvent was removed under reduced pressure and the resulting crystals were triturated with Me_2CO to afford **4h** (0.96 g, 74%). mp 137—139 °C. IR (Nujol): 3220, 1565 cm^{-1}. ^{1}H-NMR ($CDCl_3$ + DMSO-d_6 + D_2O) δ: 0.90—1.00 (m, 3H), 1.10—1.90 (m, 34H), 2.60—3.10 (m, 4H), 5.90—6.10 and 6.30—6.50 (m and m, 2H). MS *m/z*: 363 (free base M^+). *Anal.* Calcd for $C_{24}H_{46}ClNO$: C, 72.05; H, 11.59; Cl, 8.86; N, 3.50. Found: C, 72.19; H, 11.36; Cl, 8.98; N, 3.22.

2-*endo*-Hexadecylamino-5-norbornene-2,3-*exo*-dimethanol Hydrochloride (**4i**): This compound was prepared from **11n** using the same procedure as that used in the synthesis of **4h** (22% overall yield). mp 154—155 °C. IR (Nujol): 3220, 1570 cm^{-1}. ^{1}H-NMR ($CDCl_3$ + DMSO-d_6 + D_2O) δ: 0.70—1.00 (m, 3H), 1.10—2.00 (m, 29H), 2.50—3.00 (m, 4H), 3.40—4.20 (m, 4H), 5.95—6.10 and 6.40—6.55 (m and m, 2H). MS *m/z*: 393 (free base M^+). *Anal.* Calcd for $C_{25}H_{47}ClNO_2$: C, 69.98; H, 11.04; Cl, 8.26; N, 3.26. Found: C, 70.15; H, 10.81; Cl, 8.53; N, 3.18.

2-*exo*-Acetoxymethyl-2-*endo*-palmitoylamino-5-norbornene (**3g**): Ac_2O (3.17 g, 11.54 mmol) was added to a solution of **3e** (188 mg, 0.5 mmol), Et_3N (0.1 ml, 12.69 mmol) and DMAP (10 mg) in THF (3 ml) under ice cooling. The reaction mixture was stirred at room temperature for 20 h. To the mixture was added AcOEt and the solution was washed with H_2O. The organic layer was separated, dried ($MgSO_4$) and then evaporated to dryness *in vacuo* to afford **3g** as a syrup (160 mg, 76%). IR (Nujol): 3310, 1730, 1640, 1545 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.80—2.00 (m, 35H), 2.04 (s, 3H), 2.87 (br, 1H), 3.32 (br, 1H), 4.50 (s, 2H), 5.10—5.30 (m, 1H), 5.90—6.15 and 6.20—6.40 (m and m, 2H). MS *m/z*: 420 (M^+).

2-*exo*-Hexadecylamino-5-norbornene-2-*endo*-methanol Hydrochloride (**6**): This compound was prepared from **15** by the same procedure as used for the synthesis of **4h** (52% overall yield). mp 66 °C. IR (Nujol): 3600, 1465 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.70—2.10 (m, 35H), 2.50—2.950 (m, 4H), 3.23 (s, 2H), 5.90—6.35 (m, 4H). MS *m/z*: 363 (M^+). *Anal.* Calcd for $C_{24}H_{45}NO$: C, 79.27; H, 12.47; N, 3.85. Found: C, 79.51; H, 12.63; N, 3.59.

2-*endo*-Hexadecylaminonorbornane-2-*exo*-methanol Hydrochloride (**7**): Hydrogenation of compound **4h** (0.15 g, 0.37 mmol) was carried out in MeOH (10 ml) at 1 atm (H_2) over 10% palladium on charcoal (0.1 g). After a theoretical amount of hydrogen had been absorbed, the catalyst was filtered off and the filtrate was evaporated to dryness *in vacuo*. The resulting syrup was triturated with hexane to afford **7** (0.11 g, 73%). mp 119—126 °C. ^{1}H-NMR ($CDCl_3$ + DMSO-d_6 + D_2O) δ: 0.85—1.00 (m, 3H), 1.10—2.40 (m, 38H), 2.70—3.30 (m, 4H).

6-*endo*-Hydroxy-5-*exo*-iodo-2-*endo*-palmitoylaminonorbornane-2-*exo*-methanol (**5j**): I_2 (9.5 g, 37.4 mmol) was added to a solution of **13m** (7.6 g, 18.7 mmol) in CH_2Cl_2 (30 ml) and CH_3CN (50 ml). The reaction mixture was stirred at room temperature for 20 h; AcOEt was added and the solution was washed with aqueous $NaHCO_3$ and H_2O. The organic layer was separated, dried ($MgSO_4$) and then evaporated to dryness *in vacuo*. The resulting syrup was purified by column chromatography on silica gel (hexane : AcOEt = 2 : 1) to afford **16** as colorless crystals (9.3 g,

93%). mp 54—55 °C. IR (Nujol): 1735, 1665 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.80—1.90 (m, 32H), 1.90—2.30 (m, 5H), 2.50—2.70 (m, 2H), 3.45—3.55 (m, 1H), 3.69 (s, 3H), 4.95—5.05 (m, 1H). MS *m/z*: 531 (M^+). A solution of **16** (8.3 g, 15.6 mmol) in EtOH (30 ml) was added to a suspension of $Ca(BH_4)_2$ prepared from $CaCl_2$ (1.73 g, 15.6 mmol) and $NaBH_4$ (1.54 g, 40.6 mmol) in EtOH (80 ml) at −15 °C. After the reaction mixture was stirred at 5 °C for 1 h, it was poured into H_2O (400 ml). The insoluble materials were filtered and dissolved in $CHCl_3$. The solution was washed with brine, the organic layer was separated, dried ($MgSO_4$) and then evaporated to dryness *in vacuo*. The residue was purified by column chromatography on silica gel ($CHCl_3$: Me_2CO = 5 : 1) to afford **17** (6.8 g, 87%). mp 69—70 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.80—2.3 (m, 36H), 2.40—3.00 (m, 2H), 3.20—3.60 (m, 3H), 4.90—5.10 (m, 1H). MS *m/z*: 503 (M^+). Compound **17** (1.0 g, 2.0 mmol) was dissolved in 10% AcOH (50 ml) and Me_2CO (40 ml). The solution was heated at 60—70 °C for 5.5 h, $CHCl_3$ was added and the solution was washed with aqueous $NaHCO_3$. The organic layer was separated, dried ($MgSO_4$) and then evaporated to dryness *in vacuo* to afford colorless crystals. Recrystallization from AcOEt gave **5j** (0.85 g, 82%). mp 104—105 °C. IR (Nujol): 3280, 3100, 1610, 1545 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.80—1.00 (m, 3H), 1.10—2.70 (m, 34H), 3.30—3.80 (m, 3H), 4.50—4.60 (m, 1H), 4.70—4.85 (m, 1H), 5.35—5.60 (m, 1H), 8.74 (s, 1H). MS *m/z*: 503 ($M^+ - 18$).

6-*endo*-Hydroxy-2-*endo*-palmitoylaminonorbornane-2-*exo*-methanol (**5k**): Hydrogenation of **5j** (0.40 g, 0.77 mmol) was carried out in MeOH (30 ml) at 1 atm (H_2) over 10% palladium on charcoal (0.2 g) in the presence of Et_3N (0.11 ml, 0.77 mmol). After a theoretical amount of hydrogen had been absorbed, the catalyst was filtered off and the filtrate was evaporated to dryness *in vacuo*. The resulting syrup was purified by column chromatography on silica gel ($CHCl_3$: Me_2CO = 5 : 1) to afford **5k** as colorless crystals (0.3 g, 100%). mp 59—60 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.80—2.40 (m, 39H), 3.25—4.00 (m, 3H), 4.20—4.60 (m, 1H), 5.60—5.90 (m, 1H), 9.22 (s, 1H).

Enzyme Assay and Binding Assay Enzymatic activity was assayed by measuring the incorporation of ^{32}P from [γ-^{32}P]adenosine triphosphate into calf thymus HI histone using partially purified protein kinase C from rat brain cytosol, according to the method reported by Kikkawa *et al.*[15] Protein kinase A was similarly assayed using the same method. The binding of [^{3}H]PDBu to protein kinase C was determined by the method of Tanaka *et al.*[17] The results are shown in Tables I and II.

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References and Notes

1) Y. Nishizuka, *J. Am. Med. Assoc.*, **262**, 1826 (1989), and references cited therein.
2) M. Castagna, Y. Takai, K. Kaibuchi, K. Sano, U. Kikkawa, and Y. Nishizuka, *J. Biol. Chem.*, **257**, 7847 (1982); J. Yamanishi, Y. Takai, K. Kaibuchi, K. Sano, M. Castagna, and Y. Nishizuka, *Biochem. Biophys. Res. Commun.*, **112**, 778 (1983); J. E. Niedel, L. J. Kuhn, and G. R. Vandenbark, *Proc. Natl. Acad. Sci. U.S.A.*, **80**, 36 (1983).
3) R. Miyake, Y. Tanaka, T. Tsuda, K. Kaibuti, U. Kikkawa, and Y. Nishizuka, *Biochem. Biophys. Res. Commun.*, **121**, 649 (1984).
4) G. J. Mazzei, N. Katoh, and J. F. Kuo, *Biochem. Biophys. Res. Commun.*, **109**, 1129 (1982).
5) T. Tamaoki, H. Nomoto, I. Takahashi, Y. Kato, M. Morimoto, and F. Tomita, *Biochem. Biophys. Res. Commun.*, **135**, 397 (1986).
6) H. Kase, K. Iwahashi, and Y. Matsuda, *J. Antibiot.*, **39**, 1059 (1986).
7) E. Kobayashi, K. Ando, H. Nakano, and T. Tamaoki, *J. Antibiot.*, **42**, 153 (1989).
8) T. Mori, Y. Takai, R. Minakuchi, B. Yu, and Y. Nishizuka, *J. Biol. Chem.*, **255**, 8378 (1980).
9) C. J. Marasco, Jr., C. Piantadosi, K. L. Meyer, S. Morris-Natschke, K. S. Ishaq, G. W. Small, and L. W. Daniel, *J. Med. Chem.*, **33**, 985, (1990).
10) H. Hidaka, M. Inagaki, S. Kawamoto, and Y. Sasaki, *Biochemistry*, **23**, 5036 (1984).
11) *a*) Y. A. Hannun and R. M. Bell, *Science*, **235**, 670 (1987); *b*) Y. A. Hannun, C. R. Loomis, A. H. Merrill, Jr., and R. M. Bell, *J. Biol. Chem.*, **261**, 12604 (1986); *c*) Y. A. Hannun and R. M. Bell, *Science*, **243**, 500 (1989).
12) Preliminary communication: T. Iwasaki, H. Yamazaki, T. Nishitani, and T. Sato, *Chem. Pharm. Bull.*, **39**, 527 (1991).
13) H. Horikawa, T. Nishitani, T. Iwasaki, Y. Mushika, I. Inoue, and M. Miyoshi, *Tetrahedron Lett.*, **21**, 4101 (1980).
14) H. Yamazaki, H. Horikawa, T. Nishitani, T. Iwasaki, K. Nosaka, and H. Tamaki, *Chem. Pharm. Bull.*, **40**, 102 (1992).
15) U. Kikkawa, Y. Takai, R. Minakuchi, S. Inohara, and Y. Nishizuka, *J. Biol. Chem.*, **257**, 13341 (1982).
16) P. A. Crooks, P. Burn, R. D. E. Sewell, and N. Upton, *J. Pharm. Sci.*, **75**, 1010 (1986), and references cited therein.
17) Y. Tanaka, R. Miyake, U. Kikkawa, and Y. Nishizuka, *J. Biochem.* (Tokyo), **99**, 257 (1986).
18) Activation of protein kinase C by these compounds was investigated by the procedure reported by Kikkawa *et al.* See ref. 15.
19) R. Zand, O. Z. Sellinger, R. Water, and R. Harris, *J. Neurochem.*, **23**, 1201 (1974).