New Hexahydroxybiphenyl Derivatives as Inhibitors of Protein Kinase C

Yoshiki Kashiwada,† Li Huang,† Lawrence M. Ballas,‡ Jack B. Jiang,‡ William P. Janzen,‡ and Kuo-Hsiung Lee*,†

Natural Products Laboratory, Division of Medicinal Chemistry and Natural Products, School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27599, and Sphinx Pharmaceuticals Corporation, Durham, North Carolina 27717

Received May 6, 1993•

We have previously shown that some ellagitannins are potent inhibitors of protein kinase C (PKC). On the basis of this finding, several series of hexahydroxybiphenyl derivatives of ellagic acid were synthesized as simple analogs of these ellagitannins and were evaluated for their inhibitory effect against PKC. Compounds **23** and **26** were found to be potent inhibitors of PKC, while hexakis(benzyloxy)biphenyl derivatives exhibited weak anti-PKC activity.

Protein kinase C (PKC, a family of phospholipid-dependent protein kinases) plays an important role in signal transduction as well as in cellular proliferation, differentiation, and various regulatory mechanisms.[1] Since a variety of possible roles of PKC in cellular functions have been recognized, specific inhibitors of PKC might be useful as chemotherapeutic agents for human cancer[2-4] and disorders of the central nervous system,[5-7] cardiovascular system,[8-10] inflammation and immune system,[11-15] and other metabolic systems. Previously, 56 tannins were evaluated for their inhibitory effect against PKC, since known tannins, isolated from Chinese crude drugs, are potent inhibitors of PKC.[16] Among the tannins were examined, ellagitannins and complex tannins [e.g., 1(β)-*O*-galloylpedunculagin (**1**) and puricafolin (**3**)] were found to be potent inhibitors of PKC. This finding has prompted our synthesis of simpler analogs related to these compounds as new anti-PKC agents. Since these classes of tannins possess hexahydroxybiphenoyl (HHBP) group(s) as a common structural feature in their molecule, we have prepared derivatives of the HHBP group from ellagic acid and have investigated their inhibitory effect against PKC.

Chemistry

The biphenyl derivatives (**7-26**) were synthesized from ellagic acid (**5**). Ellagic acid (**5**) was benzylated according to a procedure reported by Schmidt *et al.*[17] to give tetrabenzylellagic acid (**6**). Reduction of **6** with $LiAlH_4$ yielded tetrol **7**. Further benzylation of **7** afforded the hexabenzyl compound **11**, while methylation of **7** with Me_2SO_4 and K_2CO_3 in acetone furnished the dimethyl hexabenzyl derivative **16**. Treatment of **11** and **16** with MnO_2 and $SOBr_2$ led to the formation of **12**, **17** and **13**, **18**, respectively. Alternatively, tetrabenzylellagic acid (**6**) was treated with KOH-50% EtOH, yielding the dicarboxylate. Benzylation with benzyl bromide and K_2CO_3 in dry acetone followed by hydrolysis with 2% NaOH gave the hexabenzyl dicarboxylic acid **8**, while methylation of the dicarboxylate yielded the dimethyl dimethoxy tetrahydroxy dicarboxylate **14**. Treatment of **8** with diazomethane or C_2H_5I/K_2CO_3 gave **9** and **10**, respectively. Hydrolysis of **14** with 2% NaOH followed by ethylation as for **10** furnished **15**.

In order to prepare the diamine derivatives, the dibromo derivatives (**13** and **18**) were treated with NH_4OH. However, this furnished the unexpected compounds **19** and **20**, respectively. The ^{1}H-NMR spectrum of **19** was similar to that of **8**, showing the presence of six benzyl groups [δ 6.9-7.6 (30H in total, m), 5.39, 5.29, 5.16, and 5.08 (each 2H, d, J = 12 Hz), 5.02 (4H, s)] and an aromatic singlet signal [δ 6.08 (2H, s)] ascribable to H-5 and H-5′. It also exhibited signals due to methylene groups at δ 3.58 and 2.83 (each 2H, d, J = 13 Hz), whose chemical shifts indicated the presence of aminomethyl groups. The ^{1}H-NMR spectrum of **20** was similar to that of **19**, except for the presence of four benzyl groups and two methoxy groups. However, the FAB-MS of **19** and **20** gave $(M + H)^+$ ion peaks at m/z 1647 and 1343, respectively, indicating a dimeric nature. From these observations, the structures of **19** and **20** were assigned to the symmetrical dimeric biphenyl derivatives represented by formulae **19** and **20**, respectively.

The hexabenzyl derivatives (**9-13** and **19**) were hydrogenated with Pd-C/H_2, since the hexahydroxy derivatives, which are structurally similar to the HHBP group, were expected to show strong anti-PKC activity. However, **12**, **13**, and **19** all gave a complicated mixture of products, and the desired products could not be obtained. The hydrogenation of **11** furnished a single product, which was shown by TLC examination and has been assigned structure **23a**. However, during column chromatography on Sephadex LH-20 (EtOH), only a less polar compound, **23**, was obtained. Methylation of **23** gave a hexamethylate, **25**, which was shown to be identical with 5,7-dihydro-1,2,3,9,10,11-hexamethoxydibenz[*c*,*e*]oxepine[18] by spectral comparison. Therefore, presumably the hydroxymethyl groups in **23a** formed a cyclic ether, giving 5,7-dihydro-1,2,3,9,10,11-hexahydroxydibenz[*c*,*e*]oxepine. To confirm the structure of **23**, compound **11** was treated with *p*-toluenesulfonic acid in benzene to give a 5,7-dihydrodibenz[*c*,*e*]oxepine derivative, **24**. This compound was subsequently hydrogenated with Pd-C/H_2 to furnish a hexahydroxy compound, which was identical with **23**.

Optically active biphenyl derivatives were prepared from ellagitannins [*e.g.*, pedunculagin (**2**) and corilagin (**4**)]. Thus, treatment of **2** and **4** with benzyl bromide and potassium carbonate in dry acetone followed by methanolysis with 2% NaOMe-MeOH yielded dimethyl (*S*)- and dimethyl (*R*)-3,3′,4,4′,5,5′-hexakis(benzyloxy)-1,1′-biphenoate (**9-*S*** and **9-*R***, respectively). They were treated as for the racemic compounds to furnish **10-*S***, **10-*R***, **23-*S***, **23-*R***, **24-*S***, and **24-*R***.

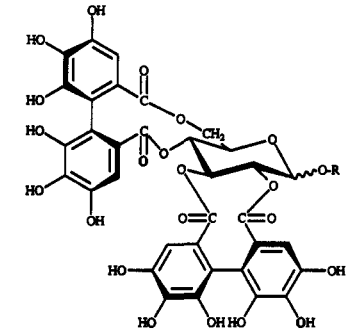
Results and Discussion

The PKC inhibitory activities for the biphenyl derivatives are summarized in Table 1. Since the phenolic

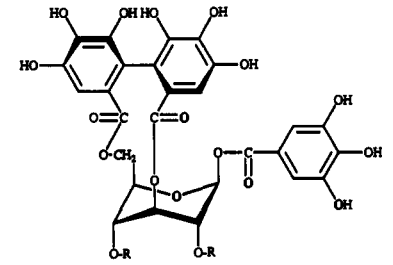
* To whom correspondence should be addressed.
† Natural Products Laboratory, School of Pharmacy, UNC-CH.
‡ Sphinx Pharmaceuticals Corporation.
• Abstract published in *Advance ACS Abstracts*, December 1, 1993.

0022-2623/94/1837-0195$04.50/0

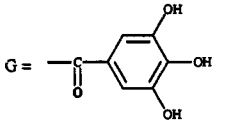
Chart 1



	R
1	G(β)
2	H



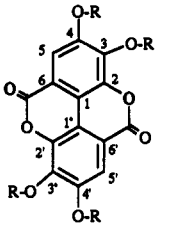
	R
3	G
4	H



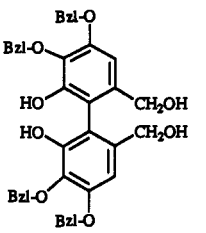
hydroxyl groups were thought to be essential for anti-PKC activity, hexahydroxylbiphenyl derivatives (**21–23** and **26**), which are structurally similar to the HHBP group, were expected to show strong anti-PKC activities. Compound **23** demonstrated potent inhibitory activity with an IC_{50} value of 43 μM against mix-PKC. It also exhibited anti-PKC activity against the α isoenzyme (IC_{50} = 37 μM). When the cyclic ether group in **23** was replaced with two dimethyl ethers as in **26**, inhibitory activity against mix- and α-PKC was also observed with IC_{50} values of 98 and 43 μM, respectively. These activities were greater than that (IC_{50} > 100 μM) of 2,3-(*S*)-HHBP-D-glucose, which has one HHBP group in the molecule. These ether groups at C-6 and C-6′ are necessary to retain anti-PKC activity. When they were replaced with methyl- or ethylcarboxyl groups as in **21** and **22**, no anti-PKC activities were found, although these compounds also possess six phenolic hydroxyl groups. This finding suggests that the number of phenolic hydroxyl groups is not important for anti-PKC activity.

Unexpectedly, a HHBP group may not be necessary for PKC inhibitory activity because the hexakis(benzyloxy)-biphenyl derivatives (**9–13**, **19**, and **25**) exhibited inhibitory activity against mix-PKC. Relatively strong anti-PKC activities were observed in **10** and **11** with IC_{50} values of 75 and 70 μM, respectively. The other hexakis(benzyloxy)-biphenyl derivatives showed weak anti-PKC activities (IC_{50} = 103–180 μM). Among the dimethoxyhexakis(benzyloxy)biphenyl derivatives, **14**, **15**, and **20** exhibited weak anti-PKC activities (IC_{50} = 94–131 μM), while no anti-PKC activities were observed in **16–18**. Thus, no definitive

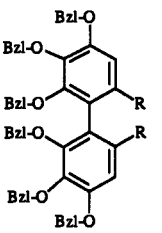
Chart 2



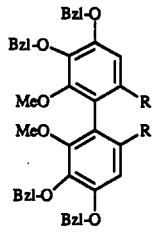
	R
5	H
6	Bzl



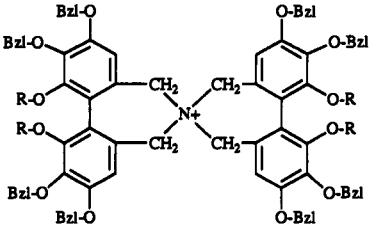
7



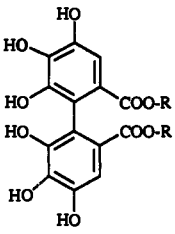
	R
8	COOH
9	COOMe
10	$COOC_2H_5$
11	CH_2OH
12	CHO
13	CH_2Br



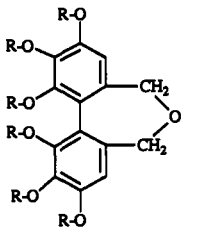
	R
14	COOMe
15	$COOC_2H_5$
16	CH_2OH
17	CHO
18	CH_2Br



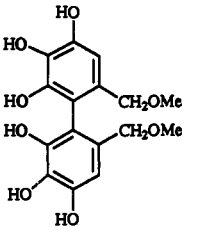
	R
19	Bzl
20	Me



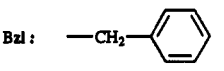
	R
21	Me
22	C_2H_5



	R
23	H
24	Bzl
25	Me



26



correlation was found between the structures and the anti-PKC activities. However, it was suggested that the compounds possessing phenolic hydroxy groups exhibited better PKC inhibitory activity.

Next, the activities for the atropisomers of compounds **9**, **10**, **11**, and **23** were compared, since atropisomerism was expected to correlate with the potency of anti-PKC activity. But again, a relationship was not found. In the case of **9** and **10**, the *S*-isomers evoked stronger activities, whiel **11-*R*** showed more potent inhibitory activity than **11-*S***. Compounds **23-*S*** and **23-*R*** demonstrated almost equivalent anti-PKC activities.

The most potent compounds (**23-*S***, **23-*R***, and **26**) were further examined for the inhibitory activities against the recombinant human PKC, β I and II, δ, and γ enzymes. Compounds **23-*S*** and **23-*R*** exhibited similar activities against β I/II-R, δ-H, and γ-H with IC_{50} values of 31, 42, and 34 μM (for **23-*S***) and 29, 44, and 30 μM (for **23-*R***), respectively. However, **26** showed inhibitory activity against γ with an IC_{50} of 50 μM, whereas no inhibition (IC_{50} > 218 μM) was obtained against δ, suggesting that the anti-PKC activity for **26** is selective.

Scheme 1

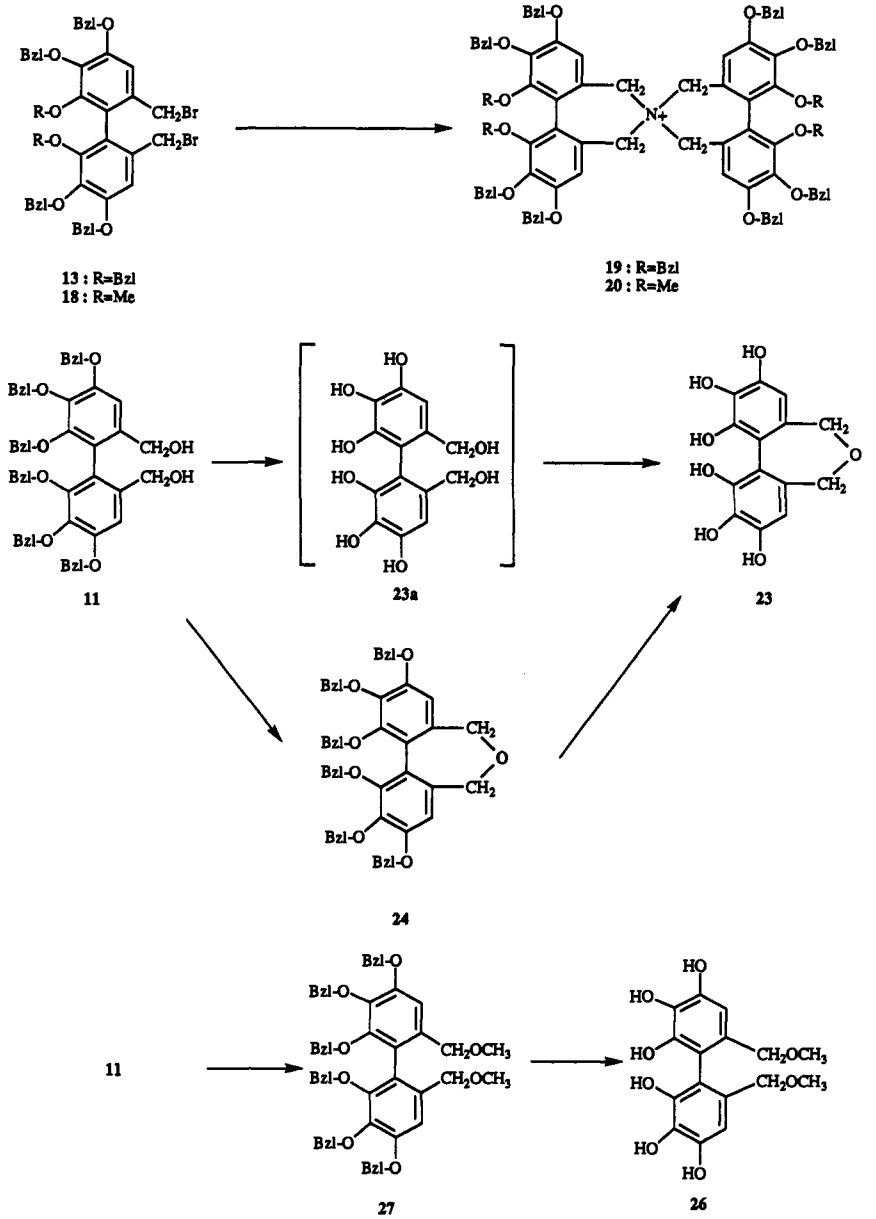


Table 1. Effect of Biphenyl Derivatives on PKC and cAMP-Dependant Protein Kinase Activity[a]

	anti-PKC activity IC_{50} (μM)	cAMP kinase IC_{50} (μM)	phorbol displacement IC_{50} (μM)		anti-PKC activity IC_{50} (μM)	cAMP kinase IC_{50} (μM)	phorbol displacement IC_{50} (μM)
1	3	>218	<50	**14**	97	–	–
2	4	>218	<50	**15**	94	–	–
3	4	>218	<50	**16**	>218	–	–
4	20	NT	NT	**17**	>218	–	–
7	>218	–	–	**18**	>218	–	–
9	180	–	–	**19**	131	–	–
9-*S*	131	–	–	**20**	87	15	>272
9-*R*	>218	–	–	**21**	>218	–	–
10	75	–	–	**22**	>218	–	–
10-*S*	50	19	>272	**23**	43	>218	54
10-*R*	96	–	–	**23-*S***	32	NT	NT
11	70	–	–	**23-*R***	28	NT	NT
11-*S*	88	–	–	**24**	180	–	–
11-*R*	55	21	>272	**25**	>218	–	–
12	131	–	–	**26**	98	>218	NT
13	103	–	–				
				Sphingosine[b]	40	NT	23

[a] Dash: not determined due to lack (>60 μM) of PKC inhibition. NT: not tested. [b] Sphingosine IC_{50} (μM) for all the PKC isoenzymes used in this study was 40–50 μM.

All of the compounds which inhibited PKC with the exception of **23** and **26** also inhibited cAMP-dependent protein kinase. This suggested that these compounds are inhibitors of the catalytic subunit of PKC. Compound **23**

did not inhibit cAMP-dependent kinase but did displace phorbol binding. This suggested that 23 is inhibiting at the regulatory site of PKC.

Experimental Section

General Experimental Procedures. All melting points were determined on a Fischer-John melting-point apparatus and are uncorrected. Optical rotations were measured with an AUTOPOL III automatic polarimeter. NMR spectra were taken with a Bruker AC-300 instrument with TMS as an internal standard, and chemical shifts are given in δ (ppm). Elemental analyses were performed by Atlantic MicroLab Inc., Norcross, GA. Column chromatography was performed with Silica 32–63 (32–63 μm, Universal Adsorbents Inc.) and Sephadex LH-20 (25–100 μm, Pharmacia). TLC was conducted on precoated Kieselgel 60 F_{254} plates (0.20 mm, Merck), and spots were detected by UV illumination and spraying with 10% H_2SO_4.

Tetrabenzylellagic Acid (6). A mixture of ellagic acid (5 g, 1.66 mmol), anhydrous potassium carbonate (19 g), potassium iodide (1 g), and benzyl chloride (20 mL, 81.3 mmol) in acetophenone (74 mL) was heated at 140 °C with stirring for 15 h. The inorganic salts and unreacted ellagic acid were removed by filtration. Tetrabenzylellagic acid (6, 3.5 g, 32% yield) crystallized from the filtrate. The physical data of 6 were identical with those described in the literature.[17]

2,2′-Dihydroxy-3,3′,4,4′-tetrakis(benzyloxy)-1,1′-biphenyl-6,6′-dimethanol (7). A suspension of 6 (1.5 g, 2.27 mmol) in THF (40 mL) was treated with $LiALH_4$ (400 mg, 10.5 mmol) at room temperature with stirring overnight. After water was added, the mixture was extracted with EtOAc. The EtOAc-soluble portion was chromatographed on silica gel [hexane–acetone (5:2 → 3:2)] to furnish 7 (1.3 g, 86% yield) as colorless needles (from hexane–acetone): mp 143–144 °C; ^{1}H NMR ($CDCl_3$) δ 7.5–7.2 (20H in total, aromatic H), 6.81 (2H, s, 5-H, 5′-H), 5.70 (2H, s, OH), 5.22 (5.10 (each 2H, d, J = 11 Hz, $PhCH_2O$), 5.20 (4H, s, $PhCH_2O$), and 4.13 (4H, s, $PhCH_2OH$). Anal. ($C_{42}H_{38}O_8$) C, H.

2,2′,3,3′,4,4′-Hexakis(benzyloxy)-1,1′-diphenyl-6,6′-dicarboxylic acid (8). A solution of 6 (4.6 g, 6.95 mmol) in 5% KOH [EtOH–H_2O (1:1)] (300 mL) was refluxed for 2 h. The reaction mixture was neutralized with 1 N HCl, and the resulting white precipitate was collected by filtration. A mixture of this precipitate, anhydrous potassium carbonate (8.0 g), and the benzyl bromide (5.0 mL, 20 mmol) in dry acetone (60 mL) was refluxed for 4 h with stirring. The reaction mixture was filtered, concentrated, and chromatographed on silica gel (benzene) to afford dibenzyl hexabenzyl dicarboxylate (4.0 g, 56% yield) as a colorless syrup: ^{1}H NMR ($CDCl_3$) δ 7.5–6.7 (42H in total, m, aromatic H), 5.14 (4H, s, $PhCH_2O$), 5.00, 4.94, 4.87, 4.82 (each 2H, d, J = 12 Hz, $PhCH_2O$), 4.75, 4.65 (each 2H, d, J = 11 Hz, $PhCH_2O$). The solution of the dibenzylate (4.0 g, 3.78 mmol) in 2% NaOH [EtOH–acetone (1:1)] (150 mL) was refluxed for 2 h. The reaction mixture was acidified with 1 N HCl and concentrated to an aqueous solution, which was extracted with EtOAc. After removal of the solvent by evaporation, the EtOAc-soluble portion was subjected to Sephadex LH-20 column chromatography. Elution with EtOH gave 8 (2.9 g, 88% yield). The physical data of 8 were identical with those described in the literature.[17]

Dimethyl 2,2′,3,3′,4,4′-Hexakis(benzyloxy)biphenyl-6,6′-dicarboxylate (9). Compound 8 (460 mg, 0.52 mmol) was treated with etheral diazomethane to give 9 (420 mg, 88% yield) as a colorless syrup: ^{1}H NMR ($CDCl_3$) δ 7.56–6.86 (32H in total, aromatic H, 5-H, 5′-H), 5.23, 4.98 (each 4H, s, $PhCH_2O$), 4.94, 4.77 (each 2H, d, J = 11 Hz, $PhCH_2OH$), 4.00 (4H, m, CH_2), 0.96 (6H, t, J = 7 Hz, CH_3).

Dimethyl (*R*)-2,2′,3,3′,4,4′-Hexakis(benzyloxy)biphenyl-6,6′-dicarboxylate (9-*R*). A mixture of corilagin (4) (2.0 g, 3.15 mmol), anhydrous potassium carbonate (5.0 g), and benzyl bromide (8.0 mL, 32.6 mmol) in dry acetone (40 mL) was refluxed for 7 h. After removal of the inorganic salt by filtration, the filtrate was concentrated to a syrup, which was applied to a silica gel column. Elution with hexane–EtOAc (2:1) furnished a fraction containing the benzyl ether, which was dissolved in 2% NaOMe–MeOH (30 mL) and left to stand overnight. The reaction mixture was neutralized with IR-120B resin, filtered, and concentrated. The residue was chromatographed on silica gel [hexane–EtOAc (5:1 → 4:1)], yielding 9-*R* (1.7 g, 66% yield) as colorless needles (from EtOH–EtOAc): mp 112–114 °C; $[\alpha]^{20}_D$ −45.1° (c = 0.63, $CHCl_3$). Anal. ($C_{56}H_{50}O_{10}$) C, H.

Dimethyl (*S*)-2,2′,3,3′,4,4′-Hexakis(benzyloxy)biphenyl-6,6′-dicarboxylate (9-*S*). Pedunculagin (2) (500 mg, 0.64 mmol) was treated as described above to give 9-*S* (690 mg, 60% yield) as colorless needles (from EtOH–EtOAc): mp 113–115 °C; $[\alpha]^{20}_D$ +50.7° (c = 0.41, $CHCl_3$). Anal. ($C_{56}H_{50}O_{10}$) C, H.

Diethyl 2,2′,3,3′,4,4′-Hexakis(benzyloxy)-1,1′-diphenyl-6,6′-dicarboxylate (10). A mixture of 8 (130 mg, 0.15 mmol), iodoethane (0.7 mL), and anhydrous potassium carbonate (700 mg) in dry acetone (10 mL) was refluxed for 2 h with stirring. After removal of the inorganic salt by filtration, the filtrate was concentrated to a syrup, which was chromatographed on silica gel. Elution with hexane–EtOAc (1:1) afforded 10 (105 mg, 76% yield) as a colorless syrup: ^{1}H NMR ($CDCl_3$) δ 7.6–6.8 (42H in total, m, aromatic H), 5.23 (m), 4.98 (each 4H, s, $PhCH_2O$), 4.94, 4.77 (each 2H, d, J = 11 Hz, $PhCH_2O$), 4.01 (4H, m, CH2), 0.96 (6H, t, J = 7 Hz, CH_3).

Diethyl (*S*)-2,2′,3,3′,4,4′-Hexakis(benzyloxy)biphenyl-6,6′-dicarboxylate (10-*S*): colorless syrup; $[\alpha]^{20}_D$ +39.0° (c = 0.39, $CHCl_3$). Anal. ($C_{60}H_{54}O_{10}$), C, H.

Diethyl (*R*)-2,2′,3,3′,4,4′-Hexakis(benzyloxy)biphenyl-6,6′-dicarboxylate (10-*R*): colorless syrup; $[\alpha]^{20}_D$ −38.0° (c = 0.51, $CHCl_3$). Anal. ($C_{60}H_{54}O_{10}$) C, H.

2,2′,3,3′,4,4′-Hexakis(benzyloxy)-1,1′-diphenyl-6,6′-dimethanol (11). A mixture of 7 (400 mg, 0.60 mmol), benzyl bromide (1.5 mL), and anhydrous potassium carbonate (2.0 g) in dry acetone (20 mL) was refluxed for 3 h with stirring. The inorganic salts were filtered, and the filtrate was concentrated to yield a syrup. This syrup was subjected to silica gel column chromatography. Elution with hexane–acetone (5:2) furnished 8 (410 mg, 81% yield) as a colorless syrup: ^{1}H NMR ($CDCl_3$) δ 7.55–6.82 (30H in total, aromatic H), 7.06 (2H, s, 5-H, 5′-H), 5.25, 5.20, 5.07, 5.02, 4.98, 4.61 (each 2H, d, J = 11 Hz, $PhCH_2O$), 4.19 (4H, s, $PhCH_2OH$), 2.72 (2H, OH).

2,2′,3,3′,4,4′-(*S*)-Hexakis(benzyloxy)-1,1′-diphenyl-6,6′-dimethanol (11-*S*): colorless syrup; $[\alpha]^{20}_D$ +91.1° (c = 0.45, $CHCl_3$). Anal. ($C_{56}H_{50}O_8$) C, H.

2,2′,3,3′,4,4′-(*R*)-Hexakis(benzyloxy)-1,1′-diphenyl-6,6′-dimethanol (11-*R*): colorless syrup; $[\alpha]^{20}_D$ −82.0° (c = 0.71, $CHCl_3$). Anal. ($C_{56}H_{50}O_8$) C, H.

Dimethyl 2,2′-Dimethoxy-3,3′,4,4′-tetrakis(benzyloxy)-1,1-diphenyl-6,6′-dicarboxylate (14). Compound 6 (1.0 g, 1.51 mmol) was hydrolyzed as for 8 to give a dicarboxylate, which was further methylated with dimethyl sulfate (1.0 mL) and anhydrous potassium carbonate (1.5 g) in dry acetone (20 mL) with refluxing for 2 h. The reaction mixture was worked up as described above and chromatographed on silica gel. Elution with hexane–acetone (5:1) furnished 14 (435 mg, 38% yield) as colorless needles (MeOH): mp 117–119 °C; ^{1}H NMR ($CDCl_3$) δ 7.5–7.3 (22H in total, m, aromatic H), 5.16, 5.15 (each 4H, s, $PhCH_2O$), 3.62, 3.59 (each 6H, s, OMe). The physical data of 14 were identical with those described in the literature.

Diethyl 2,2′-Dimethoxy-3,3′,4,4′-tetrakis(benzyloxy)-1,1′-diphenyl-6,6′-dicarboxylate (15). A solution of 14 (120 mg, 0.16 mmol) in 2% NaOH [acetone–H_2O (1:1)] (10 mL) was refluxed for 2 h. The reaction mixture was acidified with 1 N HCl and concentrated to an aqueous solution, which was extracted with EtOAc. The EtOAc-soluble portion was treated with iodoethane and anhydrous potassium carbonate in dry acetone as for 10 and furnished 15 (115 mg, 92% yield) as colorless needles (MeOH): mp 95–98 °C; ^{1}H NMR ($CDCl_3$) δ 7.5–7.2 (22H in total, m, aromatic H), 5.15, 5.12 (each 4H, s, $PHCH_2O$), 3.95 (4H, m, CH_2), 0.91 (6H, t, J = 7 Hz, CH_3). Anal. ($C_{48}H_{46}O_{10}$) C, H.

2,2′-Dimethoxy-3,3′,4,4′-tetrakis(benzyloxy)-1,1′-diphenyl-6,6′-dimethanol (16). A mixture of 7 (500 mg, 0.75 mmol), dimethyl sulfate (2.0 mL), and anhydrous potassium carbonate (3.0 g) in dry acetone (20 mL) was refluxed for 2.5 h with stirring. After removal of the inorganic salts by filtration, the filtrate was concentrated to a syrup. The syrup was subjected to silica gel column chromatography. Elution with hexane–EtOAc (3:2) gave 11 (460 mg, 88% yield) as colorless needles (from EtOH): mp 136–137 °C; ^{1}H NMR ($CDCl_3$) δ 7.47–7.24 (20H in total, aromatic H), 6.96 (2H, s, 5-H, 5′-H), 5.17, 5.11 (each 2H, d, J = 12 Hz,

$PhCH_2O$), 5.07 (4H, s, $PhCH_2O$), 4.13 (4H, s, CH_2OH), 3.63 (6H, s, OME), 2.77 (2H, OH). Anal. ($C_{44}H_{42}O_8 \cdot 1/2\ H_2O$) C, H.

Synthesis of Compounds 12 and 17. A mixture of the diol (11 or 16, 50–85 mg) and MnO_2 (200–300 mg) in dry benzene (10–20 mL) was stirred at room temperature overnight. The reaction product was filtered. The filtrate was concentrated and chromatographed on silica gel in hexane–acetone (3:1) to yield the dialdehyde (12 or 17, respectively) in 63–75% yield.

Compound 12: white amorphous powder; ^1H NMR ($CDCl_3$) δ 9.43 (2H, s, CHO), 7.55–6.83 (32H in total, aromatic H, 5-H, 5′-H), 5.25 (4H, s, $PhCH_2O$), 5.16, 5.10, 4.83, and 4.63 (each 2H, d, J = 11 Hz, $PhCH_2O$). Anal. ($C_{56}H_{48}O_8 \cdot H_2O$) C, H.

Compound 17: colorless needles (from MeOH); mp 113–115 °C; ^1H NMR ($CDCl_3$) δ 9.46 (2H, s, CHO), 7.53–7.25 (22H in total, aromatic H, 5-H, 5′-H), 5.20 (8H, s, $PhCH_2O$), 3.58 (6H, s, OMe). Anal. ($C_{44}H_{38}O_8 \cdot 1/2H_2O$) C, H.

Synthesis of Compounds 13 and 18. A mixture of the diol (11 or 16, 0.1–1.5 g) and $SOBr_2$ (0.3–1 mL) in dry benzene (10–40 mL) was stirred under ice-cooling for 2–4 h. The reaction mixture was diluted with ether, washed with water, dried over Na_2SO_4, and concentrated to a syrup. The syrup was chromatographed on silica gel [hexane–acetone (6:1) or hexane–EtOAc (6:1)] to afford the dibromide (13 or 18, respectively) in 63–72% yield.

Compound 13: colorless syrup; ^1H NMR ($CDCl_3$) δ 7.56–6.86 (30H in total, aromatic H), 7.07 (2H, s, 5-H, 5′-H), 5.22 (4H, s, $PhCH_2O$), 5.02, 4.98, 4.93, 4.80 (each 2H, d, J = 11 Hz, $PhCH_2O$), 4.20 (4H, s, $PhCH_2Br$). Anal. ($C_{56}H_{48}Br_2O_6$) C, H, Br.

Compound 18: colorless needles (from hexane–EtOAc); mp 110–112 °C; ^1H NMR ($CDCl_3$) δ 7.51–7.27 (20H in total, aromatic H), 7.01 (2H, 5-H, 5′-H), 5.16–5.10 (each 4H, s, $PhCH_2O$), 4.20, 4.13 (each 2H, d, J = 10 Hz, CH_2Br), 3.72 (6H, s, OMe). Anal. ($C_{44}H_{40}Br_2O_6$) C, H, Br.

Synthesis of 19 and 20. A solution of 13 and 18 (42–150 mg) in acetone (5 mL) was treated with a concentrated aqueous ammonia solution (28%) (3–5 mL) for 1.5–2 h at room temperature with stirring. The reaction mixture was concentrated to a syrup, which was subjected to silica gel chromatography. Elution with $CHCl_3$–MeOH–H_2O (10:1:0.1) furnished 19 and 20, respectively (56–70% yield).

Compound 19: white amorphous powder; FAB-MS m/z 1647 $(M + H)^+$; ^1H NMR ($CDCl_3$) δ 7.6–6.9 (30H in total, m, aromatic H), 6.78 (2H, s, 5,5′-H), 5.39, 5.29, 5.16, 5.03 (each 2 H, d, J = 11 Hz, $PHCH_2O$), 5.02 (4H, s, $PhCH_2O$), 3.58, 2.83 (each 2H, d, J = 13 Hz, CH_2N). Anal. ($C_{112}H_{96}NO_{12} \cdot 6.5H_2O$) C, H, N.

Compound 20: white amorphous powder; FAB-MS m/z 1343 $(M + H)^+$; ^1H NMR ($CDCl_3$) δ 7.6–7.3 (30H in total, m, aromatic H), 6.94 (2H, s, 5,5′-H), 5.32, 5.28, 5.23, 5.17 (each 2H, d, J = 11.5 Hz, $PHCH_2O$), 3.82 (6H, s, OMe), 4.14, 3.83 (each 2H, d, J = 13 Hz, CH_2N). Anal. ($C_{88}H_{80}NO_{12} \cdot 5H_2O$) C, H, N.

Hydrogenation of Compounds 9–11 and 24. A mixture of the compound (60–120 mg) and 10% Pd–C (20–40 mg) in EtOAc (8–15 mL) was stirred under a hydrogen atmosphere overnight. After removal of the catalyst by filtration, the filtrate was concentrated to a syrup, which was subjected to Sephadex LH-20 column chromatography. Elution with EtOH yielded the product (50–80% yield).

Dimethyl 2,2′,3,3′,4,4′-Hexahydroxybiphenyl-6,6′-dicarboxylate (21): colorless needles (H_2O); mp >300 °C; ^1H NMR (acetone-d_6) δ 7.10 (2H, s, 5, 5′-H), 3.45 (6H, s, OMe). Anal. ($C_{16}H_{14}O_{10} \cdot H_2O$) C, H.

Diethyl 2,2′,3,3′,4,4′-Hexahydroxybiphenyl-6,6′-dicarboxylate (22): colorless needles (H_2O); mp >300 °C; ^1H NMR (acetone-d_6) δ 7.11 (2H, s, 5, 5′-H), 3.87 (4H, q, J = 7 Hz, CH_2), 1.41 (6H, t, J = 7 Hz, CH_3). Anal. ($C_{18}H_{18}O_{10} \cdot H_2O$) C, H.

5,7-Dihydro-1,2,3,9,10,11-hexahydroxydibenz[*c,e*]oxepine (23): white powder; mp 266 °C dec; ^1H NMR (acetone-d_6) δ 7.35 (2H, s, 5, 5′-H), 4.20, 3.90 (each 2H, d, J = 11 Hz, CH_2).

5,7-Dihydro-(*R*)-1,2,3,9,10,11-hexahydroxydibenz[*c,e*]oxepine (23-*R*): white powder; mp 266 °C dec; $[\alpha]^{20}_D$ −3.4° [c = 0.32, acetone-H_2O (1:1)]. Anal. ($C_{14}H_{12}O_7$) C, H.

5,7-Dihydro-(*S*)-1,2,3,9,10,11-hexahydroxydibenz[*c,e*]oxepine (23-*S*): white powder; mp 252 °C dec; $[\alpha]^{20}_D$ +2.0° [c = 0.25, acetone-H_2O (1:1)]. Anal. ($C_{14}H_{12})_7$) C, H.

5,7-Dihydro-1,2,3,9,10,11-hexakis(benzyloxy)dibenz[*c,e*]oxepine (24). A solution of 11 (150 mg, 0.18 mmol) in dry benzene (15 mL) was refluxed in the presence of p-toluenesulfonic acid (10 mg) for 1 h. The reaction mixture was concentrated under reduced pressure to a syrup, which was subjected to silica gel column chromatography. Elution with hexane–acetone (7:2) yielded 24 (120 mg, 82% yield) as a colorless syrup: ^1H NMR ($CDCl_3$) δ 7.5–6.9 (30H in total, m, aromatic H), 6.69 (2H, s, 4, 8-H), 5.20, 5.12 (each 2H, d, J = 11.5 Hz, $PhCH_2O$), 5.14, 5.03, 5.02, 4.91 (each 2H, d, J = 11 Hz, $PhCH_2O$), 4.07, 3.54 (each 2H, d, J = 11 Hz, 5, 7-H). Anal. ($C_{56}H_{48}O_7$) C, H.

5,7-Dihydro-1,2,3,9,10,11-hexamethoxydibenz[*c,e*]oxepine (25). A mixture of 23 (25 mg, 0.086 mmol), anhydrous potassium carbonate (120 mg), and dimethyl sulfate (0.1 mL) in dry acetone (10 mL) was refluxed for 2 h with stirring. The reaction mixture was filtered, concentrated, and subjected to silica gel chromatography. Elution with hexane–EtOAc (1:1) gave 25 (21 mg, 65% yield) as colorless needles (MeOH): mp 147–148 °C; ^1H NMR ($CDCl_3$) δ 6.74 (2H, s, 4, 8-H), 4.39, 4.07 (each 2H, d, J = 11.5 Hz, 5, 7-H), 3.94, 3.92, 3.73 (each 6H, s, OMe). This compound was identical with an authentic sample as shown by spectral comparison with those described in the literature.[18]

2,2′,3,3′,4,4′-Hexahydroxy-1,1′-biphenyl-6,6′-dimethanol Dimethyl Ether (26). A mixture of 11 (250 mg, 0.29 mmol), methyl iodide (4 mL), and silver oxide (1.0 g) in dimethylformamide (5 mL) was stirred at room temperature overnight. The reaction mixture was diluted with $CHCl_3$ and filtered. The filtrate was concentrated and chromatographed on silica gel with hexane–EtOAc (4:1) to yield 2,2′,3,3′,4,4′-hexakis(benzyloxy)-1,1′-biphenyl-6,6′-dimethanol dimethyl ether (27) (220 mg, 85% yield) as a colorless syrup: ^1H NMR ($CDCl_3$) δ 7.6–6.8 (30H in total, m, aromatic H), 7.05 (2H, s, 5,5′-H), 5.22 (4H, s, $PhCH_2O$), 5.02, 4.93, 4.91, 4.80 (each 2H, d, J = 10.5 Hz, $PhCH_2O$), 4.11, 4.04 (each 2H, d, J = 12.5 Hz, CH_2O), 3.20 (6H, s, OMe). This compound (200 mg, 0.23 mmol) was subsequently hydrogenated as described above and chromatographed on Sephadex LH-20 (EtOH) to furnish 26 (40 mg, 52% yield) as colorless needles (from H_2O): mp 165–168 °C; ^1H NMR (acetone-d_6) δ 7.97, 7.42, 6.95 (each 2H, s, OH), 6.59 (2H, s, 5,5′-H), 3.99, 3.95 (each 2H, d, J = 11.5 Hz, CH_2O), 3.15 (6H, s, OMe). Anal. ($C_{16}H_{18}O_8$) C, H.

Protein Kinase C Purification. Rat brain PKC was purified as previously published.[19] The β I, β II, δ, or γ recombinant human PKC enzymes were produced using the baculovirus expression system in SF9 cells.[20] The β I, β II, δ, or γ enzymes were partially purified from the cell pellet by Polytron homogenizing the pellet in 50 mM Tris buffer, pH 8, 0.25 mM sucrose, 10 mM benzamidine, 1 mM EDTA, 1% Triton X-100, 0.2% RMSF, and 85 μM leupeptin. The homogenate was centrifuged at 19 000 rpm for 30 min in a Sorvall RC5B centrifuge. The resulting supernatant was loaded onto a 40-mL DEAE column. The PKC enzymes were eluted off the column using a linear 0–500 mM NaCl ssalt gradient. Each fraction was assayed for PKC activity, and the peal activity for each recombinant PKC was pooled and used in these studies.

Protein Kinase C Assay. PKC was assayed by quantitating the incorporation of ^{32}P from [γ-^{32}P]ATP into histone type IIIS. The reaction mixture (250 μL) contained 30 μg of phosphatidylserine (Avanti), 20 mM Hepes buffer (pH 7.5, Sigma), 10 mM $MgCl_2$, 47.5 μM EGTA, 100 μM $CaCl_2$, 200 μg/mL histone (Sigma), 10 μL of DMSO or compound in DMSO, 30 μM [^{32}P]ATP (Dupont), and the enzyme. The assay was performed for 10 min at 30 °C and terminated with 500 μL of 25% trichloroacetic acid and 100 μL of bovine serum albumin (1 mg/mL, Sigma). The reactions were filtered onto glass fiber filters and quantified by counting in a β scintillation counter. All compounds were tested at 4, 43, and 218 μM, respectively. Assay controls included a maximal lipid-activated PKC assay and a no-lipid PKC assay. The no-lipid activity was subtracted from the maximal lipid-dependent activity to account for background nonspecific kinase activities. The PKC inhibitor sphingosine, which inhibits all the PKC isoenzymes, was included as a control inhibitor for all the PKC assays.[21] The cAMP-dependent protein kinase assay and the phorbol-binding assay were performed as previously described.[16]

Acknowledgment. This investigation was supported in part by grant AI-33066 from the National Institute of Allergies and Infectious Diseases awarded to K. H. Lee.

References

(1) Nishizuka, Y. Studies and perspectives of protein kinase C. *Science* **1986**, *233*, 305–312.

(2) Gescher, A.; Dale, I. L. Protein kinase C – a novel target for rational anti-cancer drug deisgn? *Anti-Cancer Drug Des.* **1989**, *4*, 93–105.

(3) Weinstein, I. B. The origins of human cancer: molecular mechanisms of carcinogenesis and their implications for cancer prevention and treatment. *Cancer Res.* **1988**, *48*, 4135–4143.

(4) Dale, I. L.; Gescher, A. Effects of activators of protein kinase C, including bryostatins 1 and 2, on the growth of A549 human lung carcinoma cells. *Int. J. Cnacer* **1989**, *43*, 158–163.

(5) Zurgil, N.; Zisapel, N. Phorbol ester and calcium act synergistically to enhance neurotransmitter release by brain neurons in culture. *FEBS Lett.* **1985**, *185*, 257–261.

(6) Pozzan, T.; Gatti, G.; Dozio, N.; Vicentini, L. M.; Meldolesi, J. Ca^{2+}-dependent and -independent release of neurotransmitters from PC12 cells: a role for protein kinase C activation? *J. Cell Biol.* **1984**, *99*, 628–638.

(7) Publicover, S. J. Stimulation of spontaneous transmitter release by phorbol ester, 12-*O*-tetradecanoylphorbol-13-acetate, an activator of protein kinase C. *Brain Res.* **1985**, *333*, 185–187.

(8) Halenda, S. P.; Zavoico, G. B.; Feinstein, M. B. Phorbol esters and oleoyl acetoyl glycerol enhance release of arachidonic acid in platelets stimulates by Ca^{2+} ionophore A23187. *J. Biol. Chem.* **1985**, *260*, 12484–12491.

(9) Mobley, A.; Tai, H. H. Synergistic stimulation of thromboxane biosynthesis by calcium ionophore and phorbol ester or thrombin in human platelets. *Biochem. Biophys. Res. Commun.* **1985**, *130*, 717–723.

(10) Lim, M. S.; Sutherland, C.; Walsh, M. P. Phosphorylation of bovine cardiac C-protein by protein kinase C. *Biochem. Biophys. Res. Commun.* **1985**, *132*, 1187–1195.

(11) Kaibuchi, K.; Takai, Y.; Nishizuka, Y. Protein kinase C and calcium ion in mitogenic response of macrophage-depleted human peripheral lymphocytes. *J. Biol. Chem.* **1985**, *260*, 1366–1369.

(12) Guy, G. R.; Gordon, J.; Michell, R. H.; Brown, G. Synergism between diacylglycerols and calcium ionophore in the induction of human B cell proliferation mimics the inositol lipid polyphosphate breakdown signals induced by crosslinking surface immunoglobulin. *Biochem. Biophys. Res. Commun.* **1985**, *131*, 484–491.

(13) Sagi-Eisenberg, R.; Lieman, H.; Pecht, I. Protein kinase C regulation of the receptor-coupled calcium signal in histamine-secreting rat basophilic leukemia cells. *Nature* **1985**, *313*, 59–60.

(14) Katakami, Y.; Kaibuchi, K.; Sawamura, M.; Takai, Y.; Nishizuka, Y. Synergistic action of protein kinase C and calcium for histamine release from rat peritoneal mast cells. *Biochem. Biophys. Res. Commun.* **1984**, *121*, 573–578.

(15) Takahashi, I.; Nakanishi, S.; Kobayashi, E.; Nakano, H.; Suzuki, K.; Tamaoki, T. Hypercine and pseudohypercin specially inhibit protein kinase C: possible relation to their antiretroviral activity. *Biochem. Biophys. Res. Commun.* **1989**, *165*, 1207–1212.

(16) Kashiwada, Y.; Nonaka, G.; Nishioka, I.; Ballas, L. M.; Jiang, J. B.; Janzen, W. P.; Lee, K. H. Tannins as selective inhibitors of protein kinase C. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 239–244.

(17) Schmidt, O. T.; Voigt, H.; Puff, W.; Köster, R. *Justus Liebigs Ann. Chem.* **1954**, *586*, 165–178.

(18) Insole, J. M. Buttresing and electronic effects on *meta*- and *para*-methoxy substituents on the configurational stability of 5,7-dihydro-1,11-dimethoxybenz[*c*,*e*]oxepine. *J. Chem. Res., Synop.* **1990**, 378–379; *J. Chem. Res., Miniprint* **1990**, 2831–2867.

(19) Kikkawa, U.; Go, M.; Komoto, J.; Nishizuka, Y. Rapid purification on protein kinase C by high performance liquid chromatography. *Biochem. Biophys. Res. Commun.* **1986**, *135*, 636–643.

(20) Basta, P.; Strickland, M. B.; Holmes, W.; Loomis, C. R.; Ballas, L. M.; Burns, D. J. Sequence and expression of human protein kinase C-ϵ. *Biochem. Biophys. Acta* **1992**, *1132*, 154–160.

(21) Hannun, Y. A.; Loomis, C. R.; Merrill, A. H.; Bell, R. M. *Sphingosine* inhibition of protein kinase C activity and of phorbol dibutyrate binding *in vitro* and in human platelets. *J. Biol. Chem.* **1986**, *261*, 12604–12609.