JOC Article

Protein Kinase C Translocation by Modified Phorbol Esters with Functionalized Lipophilic Regions

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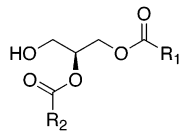
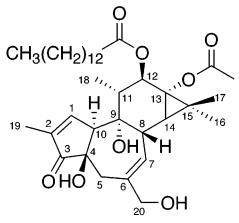
Several novel phorbol esters were prepared with polar functional groups terminating their C12 and/or C13 acyl chains. Designed to be inhibitory protein kinase C (PKC) ligands, these phorbol analogues contain various polar functional groups (amide, ester, carboxylic acid, or quaternary ammonium salt) to prevent membrane insertion of the PKC-phorbol ester complex. All phorbol derivatives were synthesized with use of diterpene starting materials obtained from croton oil, the seed oil of *Croton tiglium*. The ability of these derivatives to recruit PKC to the lipid bilayer—a usual requirement for enzyme activation—was determined by using a sucrose-loaded vesicle assay. Phorbol 12-octanoate-13-acetate derivatives translocate PKC-βII to increasing degrees as the functionality on the C12 ester becomes more hydrophobic. Likewise, PKC translocation by carboxylic acid-containing phorbol esters was dependent upon length and saturation of the hydrocarbon tether. The most promising PKC inhibitors had short carboxylic acids capping their C12 *and* C13 acyl chains, since these compounds did not recruit PKC to any appreciable extent.

Introduction

The phorbol esters are tetracyclic diterpenes isolated from the seeds of *Croton tiglium*, a member of the Euphorbiaceae (or spurge) plant family.[1] Although they have been consumed medicinally for two millennia,[2] these natural products are the most powerful tumor promoters known. For example, application of nanomolar concentrations of phorbol 12-myristate-13-acetate[3] (PMA) causes 100% of initiated mice to develop papillomas, with an average of 14 tumors per survivor.[4] In 1982, Nishizuka and co-workers discovered that phorbol esters bind to and activate protein kinase C (PKC).[5] The ubiquitious PKC family of serine/threonine kinases[6,7] plays a critical role in signal transduction pathways regulating cell growth and differentiation.[8,9] During normal signaling processes this enzyme is *transiently* activated by *sn*-1,2-diacylglycerol (DAG), which is then rapidly hydrolyzed.[10] Conversely, PMA is a stronger PKC activator[11] that is hardly metabolized by the cell.[12−14] Hence, phorbol ester binding to PKC hyperactivates the kinase, triggering cell proliferation and amplifying the efficacy of carcinogens (promotion or cocarcinogenesis).



***sn*-1,2-Diacylglycerol (DAG)**
(usually R_1 = stearoyl, R_2 = arachidonyl)



Phorbol 12-Myristate-13-acetate (PMA)

Since DAG is a competitive inhibitor of the phorbol esters,[15] it was initially thought that both ligands bind

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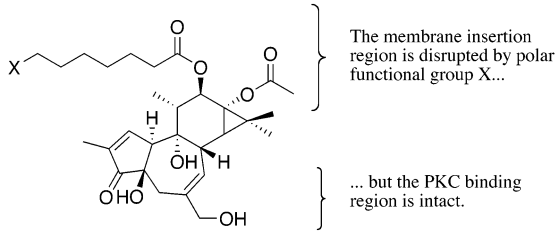
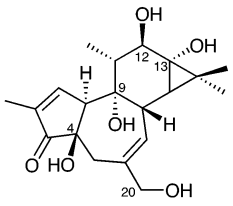
to PKC via their structurally similar *vic*-diesters.[16] A 1995 crystal structure of phorbol ester-bound PKC revealed instead that a different oxygen-rich edge of the phorbol molecule was responsible for enzyme binding. Specifically, oxygen atoms at phorbol carbons 3, 4, and 20 form a hydrogen-bonding network to three highly conserved PKC amino acid residues.[17] Twenty years ago, the laboratories of Hecker and Van Duuren established the significance of this oxygenation through structure−activity studies: synthetic modifications to C3, C4, or C20 (e.g., deoxygenation, epimerization, oxidation, reduction, or esterification) abolished or greatly diminished phorbol ester potency.[14,18] Importantly, crystallography also revealed that the phorbol C12 and C13 esters are not hydrogen-bound to PKC, and the C12 oxygen atom is found on a hydrophobic surface of the protein.[17] These data support the role of the long-chain C12 and C13 esters as lipophilic anchors facilitating membrane insertion—an absolute requirement for PKC activation[19]—of phorbol ester-bound PKC. The process of relocating inactive, cytosolic PKC to the plasma membrane is known as *membrane recruitment* or *translocation* of the kinase, and can be achieved with either phorbol esters or DAG.[11]

Phorbol esters are invaluable biochemical probes that have been used to elucidate signal transduction pathways, the mechanism of carcinogenesis, and the diverse biological activity of PKC. Due to their extremely potent cocarcinogenic activity, it is surprising that these natural products have an enormous (and largely unexplored) potential as pharmaceutical agents. For example, some naturally occurring phorbol esters are tumor *inhibitors*,[20,21] inhibit HIV replication,[22−25] or display antileukemic activity.[26,27] With this in mind, we are currently focused on the development of synthetic, phorbol-based inhibitors of PKC. Herein, we describe the preparation and PKC-recruiting activity of phorbol analogues that have polar functional groups terminating their C12 and/or C13 acyl chains. With the C3−C4−C20 binding region intact, these derivatives should remain strong PKC ligands; PKC translocation (and hence PKC activation) by these phorbol esters is expected to diminish because the added hydrophilic functionality will not insert well into the lipid bilayer. These novel phorbol esters are therefore a rationally designed class of PKC inhibitors, because they should bind strongly to the enzyme without activating it.



Results

Synthesis of Phorbol Derivatives. The most potent tumor-promoting phorbol esters are 12,13-diesters with unsubstituted 4, 9, and 20 hydroxyls. Phorbol, the parent diterpene of phorbol esters, contains five hydroxy groups with different reactivities toward acylation.[1] Due to accessibility differences, the alcohols are acylated in the following order: 20-OH > 13-OH > 12-OH ≫ 9-OH ≈ 4-OH. To selectively acylate the C12 or C13 hydroxyls, the primary allylic alcohol at C20 must first be protected. Possible phorbol 20-OH protecting groups include simple aliphatic esters,[28,29] triphenylmethyl ethers,[30] and silyl ethers.[31] We first employed phorbol 20-(*p*-methoxy)trityl ether as a starting material because it is commercially available (albeit very expensive) and the mono(*p*-methoxy)trityl (MMTr) protecting group is easily removed in 80% acetic acid.[32] Since several of the phorbol ester derivatives in this study contain free carboxylic acids, concentration often resulted in the intermolecular removal of the MMTr group (see Supporting Information). Instead, the more robust triphenylmethyl (Tr) ether was used due to its greater stability toward carboxylic acids and silica gel chromatography. Substitution at the 20-position also prevents autoxidation of phorbol, a problem commonly encountered when the C20 hydroxyl is unprotected.[33]



Phorbol

There are several published protocols[1,34,35] for the isolation of phorbol from hydrolyzed croton oil; however, its crystallization is slow and decomposition often occurs

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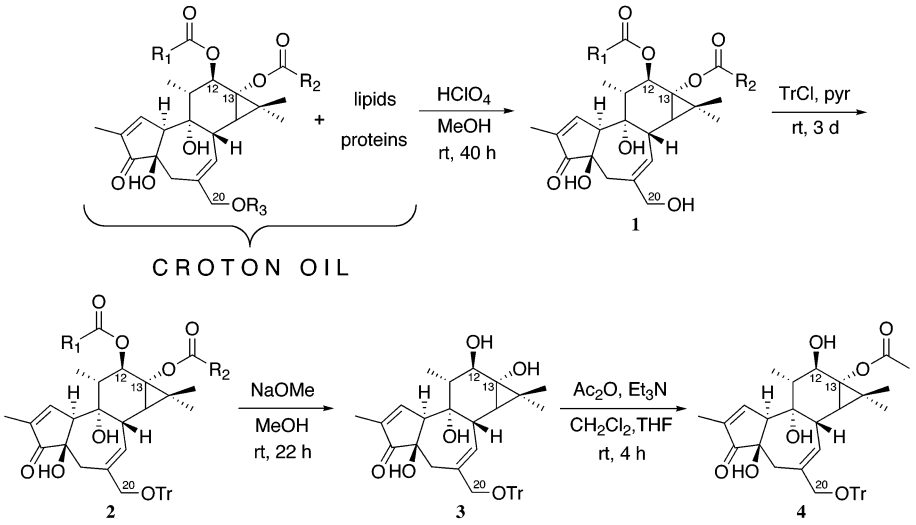
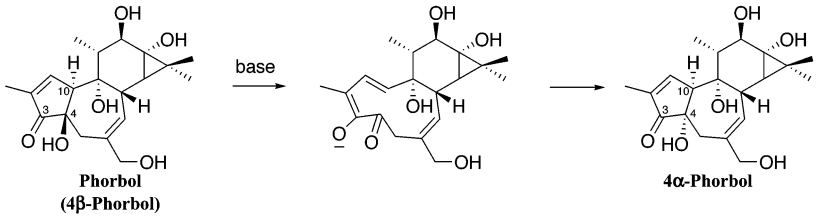
SCHEME 1. Isolation of Tritylated Phorbol Esters from Croton Oil[a]



[a] R_1 and R_2 are different alkyl groups. R_3 is a proton or an acyl group.

SCHEME 2. Base-Catalyzed C4 Epimerization of Phorbol[1]



before satisfactory crystals are obtained. Fortunately, Dr. Bernd Sorg of the Deutsches Krebsforschung Zentrum graciously gave us his procedure for the isolation of tritylated phorbol derivatives (Scheme 1). Croton oil, which contains various phorbol 12,13-diesters and phorbol 12,13,20-triesters, was treated with dilute perchloric acid in methanol to transesterify the C20 esters. The resulting phorbol 12,13-diesters (**1**) were then protected at 20-OH by using trityl chloride in pyridine, giving a complex mixture of 20-tritylated phorbol 12,13-diesters (**2**). Hydrolysis of **2** with sodium methoxide produced phorbol 20-trityl ether (**3**), which was selectively acetylated at the 13-OH to yield phorbol 13-acetate-20-trityl ether (**4**).[30] **3** can be purified by silica gel chromatography and **4** is a highly crystalline solid. Using this protocol, 1.6 g of pure **4** were obtained from 100 g of croton oil.

Acylations of phorbol have traditionally been conducted with acid chlorides in pyridine,[36] but these reactions are often low-yielding and slow. Instead, C12 and/or C13 esterifications of phorbol were performed with 10 equiv of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI),[37] 10 equiv of the desired carboxylic acid, and a stoichiometric amount of 4-(dimethylamino)pyridine (DMAP). All esterifications were purified via chromatography on silica gel, and yields were consistently good (71−96%). Allyl esters[38] were employed to protect the carboxylic acid-containing phorbol derivatives. Removal of the allyl moiety was accomplished with tetrakis(triphenylphosphine)palladium(0) and morpholine in yields dependent upon catalyst quality (26−99%). A 10% solution of TFA in anisole at 0 °C was used to remove the trityl ether. The structures of the novel phorbol derivatives prepared in this study are summarized in Table 1, and their syntheses are included in the Supporting Information.

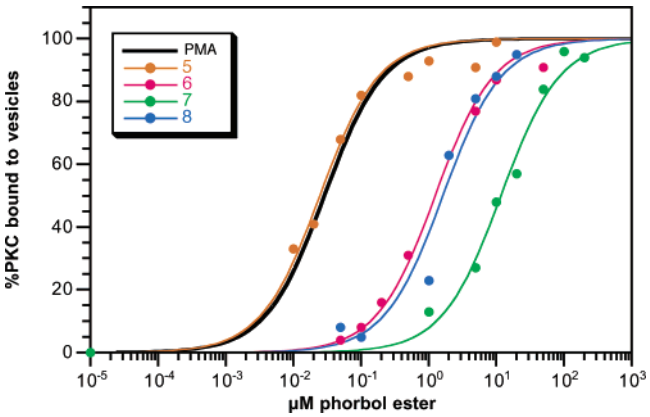


FIGURE 1. PKC recruitment by phorbol derivatives with different functional groups.

An artifact of phorbol chemistry is the inevitable contamination of phorbol ester samples with 2−5% of a diastereomeric impurity. When exposed to organic or inorganic bases, phorbol esters epimerize to their 4α-epimer[39,40] via a thermodynamically favorable rearrangement (Scheme 2). Since the undesirable 4α-epimer is difficult to separate by silica gel chromatography, all final

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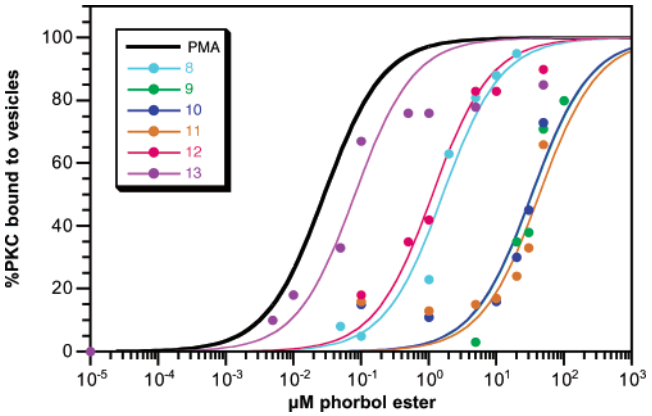


FIGURE 2. PKC recruitment by phorbol derivatives with different tether lengths. The binding curves for **9** and **10** are nearly identical.

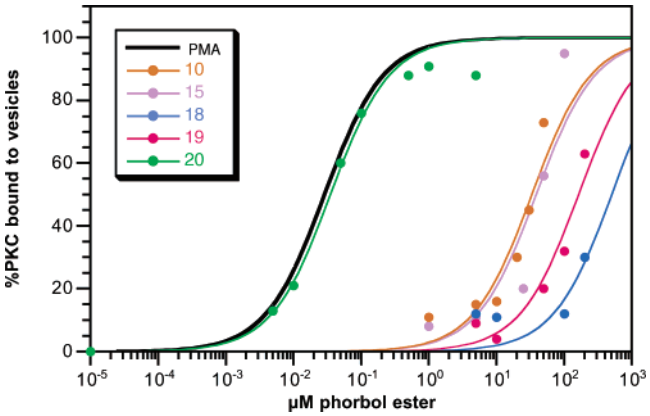
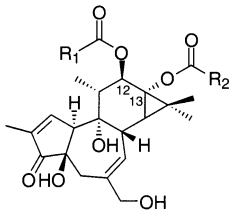


FIGURE 3. PKC recruitment by phorbol derivatives with aromatic versus aliphatic acids.

TABLE 1. Apparent K_D for Membrane Binding of Phorbol Ester Derivatives[a]



compd	R_1	R_2	K_D (μM)
5	$-(CH_2)_6CO_2CH_3$	$-CH_3$	0.024 ± 0.003
6	$-(CH_2)_6CONH_2$	$-CH_3$	1.3 ± 0.1
7	$-(CH_2)_7N(CH_3)_3^+ I^-$	$-CH_3$	12 ± 1
8	$-(CH_2)_6CO_2H$	$-CH_3$	1.6 ± 0.3
9	$-CO_2H$	$-CH_3$	34 ± 7
10	$-(CH_2)_2CO_2H$	$-CH_3$	33 ± 6
11	$-(CH_2)_4CO_2H$	$-CH_3$	43 ± 9
12	$-(CH_2)_8CO_2H$	$-CH_3$	1.1 ± 0.2
13	$-(CH_2)_{12}CO_2H$	$-CH_3$	0.024 ± 0.002
14	$-(CH_2)_2CH{=}CH-(CH_2)_2CO_2H$	$-CH_3$	19 ± 2
15	$-(p-C_6H_4)CO_2H$	$-CH_3$	39 ± 13
16	$-(p-C_6H_4)CO_2H$	$-(p\text{-}C_6H_4)CO_2H$	500 ± 200
17	$-(CH_2)_2CO_2H$	$-(CH_2)_2CO_2H$	700 ± 300
18	$-CH_3$	$-(CH_2)_2CO_2H$	500 ± 200
19	$-CH_3$	$-(p\text{-}C_6H_4)CO_2H$	160 ± 20
20	$-(CH_2)_{12}CH_3$	$-(CH_2)_2CO_2H$	0.035 ± 0.005
21	$-CH_3$	$-(CH_2)_{12}CO_2H$	0.24 ± 0.02

[a] PMA was found to have a K_D value of 0.025 ± 0.004 μM.

products were purified to >99% purity via reverse-phase HPLC. Removal of 4α-phorbol 12,13-diesters is critical for biological studies because 4α-phorbol derivatives do not activate PKC.[14]

PKC Translocation Studies of Phorbol Derivatives. Phorbol ester analogues were assayed for their ability to translocate PKC-βII to lipid vesicles by using a sucrose-loaded vesicle assay (see Experimental Section). Membrane recruitment of PKC-βII by these derivatives is shown graphically in Figures 1−6. Each figure contains a binding curve for PMA with a K_D value of 0.025 ± 0.004 μM PMA.[41] The apparent K_D for membrane binding—the concentration of phorbol ester required to translocate 50% of PKC to the lipid bilayer—for each phorbol derivative is also listed in Table 1. The PKC-catalyzed substrate phosphorylation (activity) was also measured (see Experimental Section). The PKC activity observed in the presence of either PMA or analogue **8** is shown in Figure 7. Activity curves mimic binding curves with half-maximal activation at roughly the same value as the K_D shown in Table 1. Half-maximal activation is at 15 and 800 nM for PMA and analogue **8**, vs K_D values of 25 and 1600 nM, respectively. Table 2 shows the percentage of PKC activity obtained at the K_D of each remaining

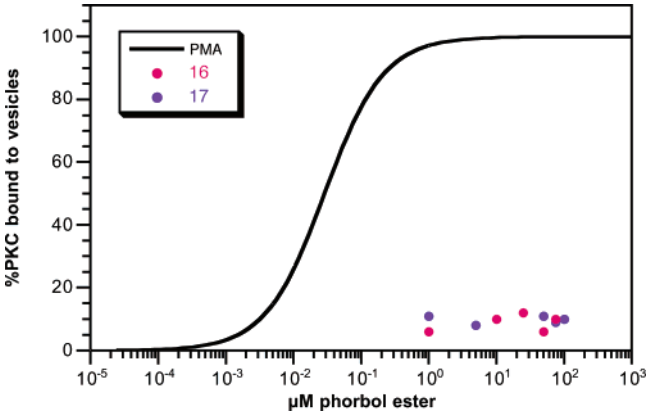


FIGURE 4. PKC recruitment by diacid phorbol derivatives.

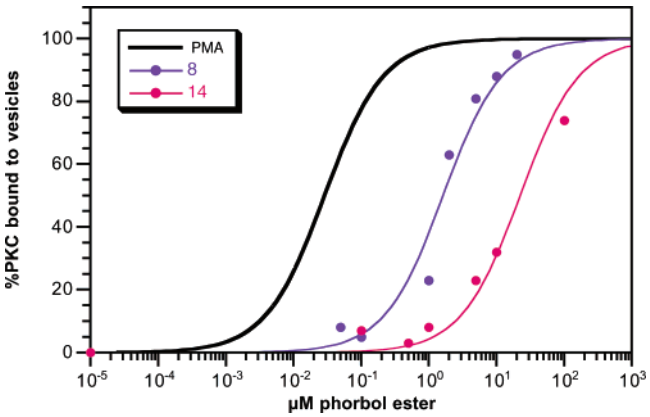


FIGURE 5. Effect of unsaturation on PKC recruitment.

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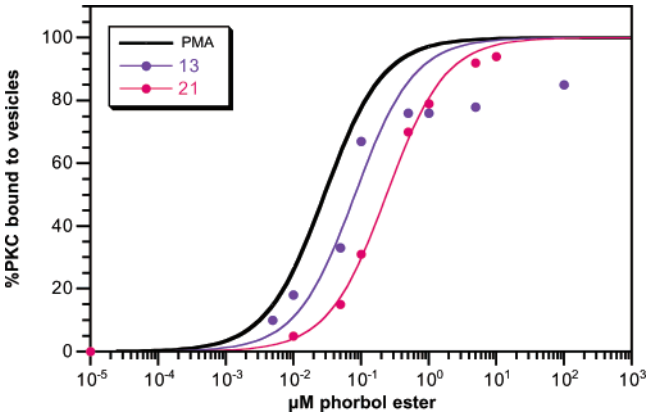

FIGURE 6. Nonequivalence of C12 and C13 esters.

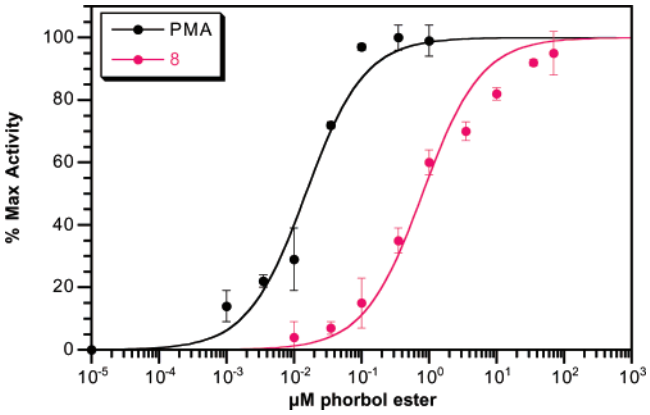

FIGURE 7. PKC activity in the presence of PMA or acid **8**.

TABLE 2. Percent PKC Activity at the Apparent K_D for Membrane Binding of Phorbol Ester Derivatives

compd	% max activity	compd	% max activity
5	59 ± 4	**14**	62 ± 1
6	50 ± 2	**15**	54 ± 9
7	70 ± 2	**16**	0
9	67 ± 6	**17**	0
10	41 ± 3	**18**	63 ± 6
11	31 ± 7	**19**	31 ± 8
12	61 ± 10	**20**	51 ± 1
13	26 ± 3	**21**	38 ± 5

analogue. Figure 8 shows the relative activity obtained with high concentrations of analogue **17**. Activity is shown relative to vesicles containing no analogue. PMA (1 nM) was included in all vesicles in Figure 8.

Discussion

A series of phorbol 12-octanoate-13-acetates (compounds **5**−**8**) were synthesized based on natural phorbol esters, which always contain one short and one long acyl group.[42] These analogues are identical to each other except for the functional group substitution at the C12 ester. Redistribution of PKC to the lipid bilayer diminishes as these functional groups increase in polarity (Figure 1): methyl ester **5** displays similar activity to PMA, amide **6** and acid **8** recruit PKC at 1 μm concentrations, and quaternary ammonium salt **7** is less effective than PMA by about 2 orders of magnitude.

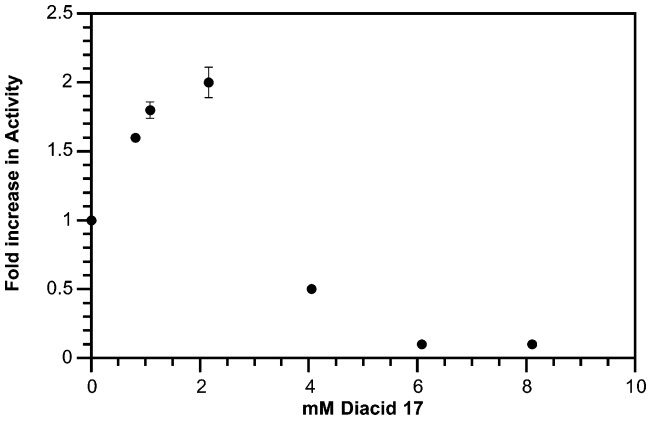

FIGURE 8. Relative PKC activity at high concentrations of diacid **17**.

Another set of analogues (**8**−**13**) with the *same* functional group—a carboxylic acid—were only different due to the length of their C12 acyl chains (Figure 2). These phorbol derivatives became more efficient at translocating PKC as their C12 side chains increased in length. Although none can recruit PKC to the lipid bilayer more efficiently than PMA, the more potent phorbol acids **12** and **13** resemble PMA in structure. Indeed, the strongest known phorbol ester promoters have C12 and C13 acyl groups with a combined total of 14 to 20 carbon atoms[43] (such as PMA, which has 16).

At cytosolic pH, carboxylic acids equilibrate between unprotonated and protonated states but only the latter is appreciably lipophilic. Benzoic diacids are about an order of magnitude more acidic than aliphatic acids[44] and therefore more likely to be ionized at physiological pH. As shown in Figure 3, we compared PKC recruitment by phorbol esters with aromatic (**15** and **19**) versus aliphatic acids (**10** and **18**). No difference in PKC translocation was observed between **10** and **15**; surprisingly, **19** (a constitutional isomer of **15**) very poorly translocated PKC and **18** (a constitutional isomer of **10**) did not recruit PKC at all. Incidentally, when the 12-acetate moiety of **18** is replaced with tetradecanoate (**20**), PKC translocation is completely restored. The presence of *two* short carboxylic acids, whether aromatic or aliphatic, in the lipophilic region of a phorbol ester is sufficient to prevent any translocation of PKC to lipid vesicles. As shown in Figure 4, phorbol diacids **16** and **17** are completely unable to membrane-recruit PKC even at 0.1 mM concentrations.

Unsaturation of the C12 and C13 acyl groups impairs both the cocarcinogenic activity[45,46] and PKC binding[47] of a phorbol ester. To examine the effect of unsaturation

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on PKC recruitment, we compared the activity of two phorbol analogues (**8** and **14**) that were only different by one degree of unsaturation. As shown in Figure 5, the presence of a single *Z*-alkene in compound **8** diminishes membrane recruitment by more than an order of magnitude. Conceivably, it is the conformational rigidity imposed by the added alkene that encumbers translocation of the phorbol−PKC complex.

Since phorbol esters bind to PKC within the chiral milieu of the enzyme, substitution of a carboxylic acid at the C12 *or* C13 acyl chain might produce two phorbol esters with unique biological activities. As shown in Figure 6, phorbol 12-tetradecandioate-13-acetate (**13**) is about three times more strongly recruiting than phorbol 12-acetate-13-tetradecandioate (**21**). After also comparing constitutional isomers **10** versus **18** and **15** versus **19** (Figure 3), we consistently observed that phorbol derivatives with free carboxyl groups on the C12 acyl chain were several times more potent than their constitutional isomers.

The ability of phorbol analogues to translocate PKC to the membrane varies according to the hydrophobicity of their side chains, and the ability of the compounds to incorporate into the membrane. Analogues that do not translocate PKC well are likely not incorporated into the membrane to a full extent.

Our studies do not address whether the analogues directly bind to PKC either in solution or while in the membrane. However, the basic structure of the phorbol molecule was not changed, and all binding sites for PKC were unaltered. Since most of the analogues could recruit PKC to the membrane at least partially, this suggests that they could in fact bind directly to the enzyme while partitioned in the membrane. While in solution, there is an orientation issue. It would be much less likely for PKC to bind to the analogues in the correct way, since they are not presented on a 2-dimensional membrane, but rather in 3-dimensional solution. Also, the concentration of analogues sensed by PKC in solution would be much lower than the concentration in a membrane. Therefore, the amount of soluble phorbol ester required to bind PKC would be orders of magnitude higher in solution. Any phorbol inhibitor would have to be present in much higher amounts than the competing membrane-bound phorbol ester.

Since most of the analogues tested were still able to recruit PKC to the membrane, it was not surprising that their relative strengths at activating the enzyme coincided with this (Figure 7 and Table 2). Half-maximal activity was seen at about the same value as the measured K_D for binding. Porbol diacids **16** and **17** did not activate PKC at their measured K_D. Slight activation was observed at 0.8−2 mM, followed by complete inhibition at higher concentrations (Figure 8). A promising possibility is that the unincorporated analogue is able to bind to PKC in solution and thus prevent PKC from binding to the membrane. However, the high concentration required for inhibition could reflect nonspecific effects of the compound on the kinase activity since such high concentrations cause some inhibition using a cofactor-independent activity assay (data not shown). Nonetheless, these data suggest some promise for the ability to design translocation inhibitors that trap PKC away from its membrane activators. In a recent study, Wada et al. synthesized a phorbol ester analogue with a hydrophilic 12-ester chain.[48] This compound was able to inhibit PKC activity by about 30%, and could compete with PMA for binding to PKC. Its effect on membrane translocation of PKC was not tested, nor were potential inhibitory effects of the compound on the direct catalytic activity of PKC assessed. Nonetheless, this study supports the potential for the design of translocation inhibitors as a mechanism for preventing PKC activation.

Conclusion

The phorbol ester lipophilic domain, largely comprised of the C12/C13 diacyl moiety, is thought to be responsible for redistributing the PKC−phorbol complex to the plasma membrane. Crystallographic data show that a large, exposed area of the phorbol-binding domain of PKC contains only uncharged, nonpolar amino acid residues. Phorbol ester binding is thought to create a continuous hydrophobic region at this surface that facilitates membrane association of the PKC−phorbol ester complex.[17] Apparently, membrane insertion of this complex is so thermodynamically favorable that the energetic cost of introducing a single hydrophilic functional group can be overcome. The presence of two short carboxylic acids, however, is sufficient to abolish PKC translocation altogether, and when present at very high concentrations can inhibit up to 90% of the measured PKC activity. Thus, we have shown the ability of a phorbol ester to membrane-recruit PKC can be gradually decreased or completely abolished depending on the polarity and amount of functional group substitution. These data are entirely consistent with the role of the C12 and/or C13 acyl chains as lipophilic anchors that permit membrane-insertion of phorbol ester-bound PKC.

Experimental Procedures

Caution! Phorbol esters are powerful tumor promoters that are rapidly absorbed through the skin. They are also severely irritating to skin, eyes, mucous membranes, and lungs. Care should be exercised when handling croton oil and all phorbol ester derivatives.

General Methods. Due to the air-sensitivity of phorbol esters, reactions were degassed via 3 freeze−pump−thaw cycles and then conducted under nitrogen. Phorbol ester derivatives were stored in CH_2Cl_2 or ethyl acetate[33] and never chloroform nor ethereal solvents. All reagents were obtained from commercial suppliers and used as received unless otherwise indicated. Tetrahydrofuran (THF) was glass-distilled from potassium and benzophenone ketyl under a nitrogen atmosphere. Dimethylformamide (DMF) and methylene chloride (CH_2Cl_2) were glass-distilled from calcium hydride under a nitrogen atmosphere. Melting points are uncorrected. Mass spectrometry was performed by the Scripps Research Institute in La Jolla, CA. Reverse-phase HPLC was performed on a semipreparative C8 column with a photodiode array detector at 254 nm; samples were dissolved in 200 μL of 50% MeOH and centrifugally filtered (0.45 μm nylon).

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Isolation of Phorbol Starting Materials 3 and 4 from Croton Oil. Croton oil (100 g, Sigma) was added to 350 mL of a 0.01 M $HClO_4$ solution in MeOH and vigorously stirred so that a brown emulsion formed. After 40 h, this mixture was neutralized with 1 g of NaOAc and partitioned between hexanes and MeOH. The MeOH layer was washed with hexanes (3 × 500 mL) and concentrated in vacuo to give a reddish-brown syrup. The syrup was dissolved in 100 mL of pyridine and 30 g of trityl chloride was added. The solution became a cloudy beige color and was stirred for 72 h, upon which it was concentrated in vacuo and partitioned between EtOAc and saturated aqueous $NaHCO_3$. The organic layer was washed successively with saturated aqueous $NaHCO_3$ (400 mL), 1:1 brine:1.0 M HCl (400 mL), and saturated aqueous $NaHCO_3$ (400 mL). Concentration of the organic layer in vacuo gave an orange paste that was triturated with MeOH (150 mL) and filtered to remove precipitated triphenylmethanol. To the clear, amber filtrate was added portionwise 0.81 g of NaOMe and the reaction was stirred for 22 h. Addition of a 10% solution of AcOH in MeOH to achieve neutrality, concentration in vacuo, and chromatography on silica gel (using an eluant gradient of 10% acetone in CH_2Cl_2, EtOAc, and 1:1 acetone:EtOAc) gave 1.85 g of **phorbol 20-trityl (3)** as an off-white resin. A small sample (isolated as a hydrate) was purified by RP-HPLC (65% MeOH in H_2O, t_R = 19.0 min) for characterization. $[\alpha]_D$ +3.3° (*c* 0.6, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.56−7.53 (m, 1H), 7.42 (d, J = 7.3 Hz, 6H), 7.29 (t, J = 7.3 Hz, 6H), 7.23 (t, J = 7.2 Hz, 3H), 5.55 (d, J = 4.0 Hz, 1H), 4.14−4.10 (m, 1H), 3.59 (AB, J = 12.5 Hz, 2H), 3.04 (t, J = 5.1 Hz, 1H), 2.92−2.87 (m, 1H), 2.45 (d, J = 20.5 Hz, 1H), 2.35 (d, J = 19.0 Hz, 1H), 2.12 (s, 1H), 1.86 (dq, J = 11.0, 6.4 Hz, 1H), 1.79−1.76 (m, 3H), 1.32 (s, 3H), 1.19 (s, 3H), 1.07 (d, J = 6.6 Hz, 3H), 0.80 (d, J = 5.1 Hz, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 208.3, 159.7, 143.9, 139.0, 133.0, 128.7, 128.5, 127.7, 126.9, 87.0, 81.5, 78.6, 73.6, 68.8, 62.9, 57.6, 45.2, 39.5, 39.0, 36.2, 26.8, 23.4, 17.1, 15.2, 10.3 ppm; IR (neat) 3412, 1697, 1628 cm^{-1}.

Following a literature procedure,[30] phorbol 20-trityl (1.85 g, 3.05 mmol) was dissolved in CH_2Cl_2 (120 mL) and THF (120 mL) and to this mixture were sequentially added Et_3N (14 mL) and Ac_2O (8 mL). After 4 h the reaction was quenched with saturated aqueous $NaHCO_3$ (200 mL), stirred 1 h, and partitioned between EtOAc and saturated aqueous $NaHCO_3$. The organic layer was washed successively with saturated aqueous $NaHCO_3$ (200 mL), 1:1 brine:0.1 M HCl (200 mL), and saturated aqueous $NaHCO_3$ (200 mL). Concentration of the organic layer in vacuo and recrystallization from warm CH_2Cl_2 gave 1.61 g (2.48 mmol, 81%) of **phorbol 13-acetate-20-trityl (4)** as a white solid. For characterization, a small sample was purified by RP-HPLC (65% MeOH in H_2O, t_R = 19.0 min) to give white crystals, mp 219−220 °C; $[\alpha]_D$ +8.4° (*c* 0.6, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58−7.56 (m, 1H), 7.42 (d, J = 7.3 Hz, 6H), 7.29 (t, J = 4.0 Hz, 6H), 7.23 (t, J = 7.2 Hz, 3H), 5.60 (d, J = 4.4 Hz, 1H), 3.99 (dd, J = 9.9, 3.3 Hz, 1H), 3.56 (s, 2H), 3.11 (t, J = 5.1 Hz, 1H), 3.07 (br s, 1H), 2.49 (d, J = 19.0 Hz, 1H), 2.37 (d, J = 19.0 Hz, 1H), 2.13 (s, 3H), 2.04 (s, 1H), 1.98 (dq, J = 10.4, 6.4 Hz, 1H), 1.80−1.77 (m, 3H), 1.27 (s, 3H), 1.23 (s, 3H), 1.09 (d, J = 5.1 Hz, 3H), 1.02 (d, J = 6.6 Hz, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 208.4, 173.9, 160.1, 143.8, 138.6, 132.8, 128.8, 128.5, 127.7, 126.9, 86.9, 78.2, 77.4, 73.6, 69.0, 68.4, 56.9, 45.0, 39.3, 39.1, 35.4, 26.8, 23.8, 21.2, 17.0, 15.3, 10.3 ppm; IR (neat) 3386, 1720, 1695 cm^{-1}; LRMS (ES) 671 (MNa^+, 100) 243 (13); HRMS (MALDI) calcd for $C_{41}H_{44}O_7Na$ (MNa^+) 671.2985, found 671.2999.

Phorbol 12-(methyl suberate)-13-acetate (5). White film, mp 28.5 °C dec; $[\alpha]_D$ +14.7° (*c* 0.2, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.59 (s, 1H), 5.68 (d, J = 5.7 Hz, 1H), 5.54 (br s, 1H), 5.40 (d, J = 10.4 Hz, 1H), 4.01 (AB, J = 12.0 Hz, 2H), 3.66 (s, 3H), 3.24−3.23 (m, 2H), 2.51 (AB, J = 7.7 Hz, 2H), 2.31 (apparent quartet, J = 6.9 Hz, 4H), 2.14 (dq, J = 10.4, 6.0 Hz, 1H), 2.09 (s, 3H), 1.78−1.77 (m, 3H), 1.66−1.58 (m, 6H), 1.36−1.31 (m, 4H), 1.23 (s, 3H), 1.20 (s, 3H), 1.09 (d, J = 2.6 Hz, 1H), 0.88 (d, J = 6.3 Hz, 3H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 208.5, 173.9, 173.5, 173.3, 160.4, 140.3, 132.7, 128.9, 78.1, 76.6, 73.7, 68.0, 65.6, 56.2, 51.6, 43.0, 39.1, 38.7, 36.3, 34.6, 34.0, 28.9, 28.7, 25.7, 25.0, 24.9, 24.0, 21.2, 17.0, 14.6, 10.3 ppm; IR (neat) 3410, 1723, 1653 cm^{-1}; HRMS (MALDI) calcd for $C_{31}H_{44}O_{10}Na$ (MNa^+) 599.2832, found 599.2831.

Phorbol 12-*O*-Suberamide-13-acetate (6). Clear oil; $[\alpha]_D$ +5.0° (*c* 0.3, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (br s, 1H), 5.68 (d, J = 4.9 Hz, 1H), 5.51 (br s, 1H), 5.41 (d, J = 10.3 Hz, 1H), 5.40 (br s, 2H), 4.02 (AB, J = 13.0 Hz, 2H), 3.24 (s, 1H), 2.70 (s, 1H), 2.56 (d, J = 19.0 Hz, 1H), 2.48 (d, J = 19.0 Hz, 1H), 2.34 (t, J = 7.3 Hz, 2H), 2.21 (t, J = 7.6 Hz, 2H), 2.14 (dq, J = 10.3, 6.3 Hz, 1H), 2.09 (s, 3H), 2.05 (br s, 1H), 1.78−1.70 (m, 3H), 1.68−1.58 (m, 4H), 1.39−1.33 (m, 4H), 1.24 (s, 3H), 1.21 (s, 3H), 1.09 (d, J = 5.4 Hz, 1H), 0.90 (d, J = 6.3 Hz, 3H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 208.6, 176.2, 173.5, 173.2, 160.4, 140.4, 132.8, 128.8, 78.1, 76.8, 73.7, 68.0, 65.6, 56.2, 43.0, 39.1, 38.6, 36.3, 35.7, 34.5, 28.9, 28.6, 25.8, 25.3, 25.0, 24.0, 21.2, 17.0, 14.7, 10.3 ppm; IR (neat) 3373, 3223, 1710, 1663 cm^{-1}; HRMS (MALDI) calcd for $C_{30}H_{43}NO_9Na$ (MNa^+) 584.2835, found 584.2826.

Phorbol 12-[8-(Trimethylammonium)octanoate]-13-acetate Iodide (7). Off-white powder, mp 243.5 °C dec; R_f 0.16 (8:6:6:3 $CHCl_3$:MeOH:acetone:concd NH_4OH); $[\alpha]_D$ +12.3° (*c* 1.1, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 7.55−7.54 (m, 1H), 5.62 (d, J = 4.4 Hz, 1H), 5.44 (d, J = 10.3 Hz, 1H), 3.94 (AB, J = 13.0 Hz, 2H), 3.37−3.30 (m, 2H), 3.14 (s, 9H), 2.51 (AB, J = 18.9 Hz, 2H), 2.44−2.32 (m, 2H), 2.21 (dq, J = 10.4, 6.4 Hz, 1H), 2.06 (s, 3H), 1.83−1.76 (m, 2H), 1.74 (dd, J = 2.8, 1.3 Hz, 3H), 1.66 (pentet, J = 7.3 Hz, 2H), 1.42 (br s, 6H), 1.25 (s, 3H), 1.21 (s, 3H), 1.16 (d, J = 5.1 Hz, 1H), 0.88 (d, J = 6.2 Hz, 3H) ppm; ^{13}C NMR (100 MHz, CD_3OD) δ 210.5, 175.7, 175.4, 160.7, 143.0, 134.7, 129.4, 79.8, 78.4, 74.7, 68.0, 67.8, 67.1, 53.6, 52.3, 44.3, 40.0, 38.4, 37.0, 35.2, 29.8, 27.22, 27.15, 25.9, 24.1, 23.8, 21.0, 17.3, 14.8, 10.2 ppm; IR (KBr) 3396, 1708, 1631 cm^{-1}; HRMS (MALDI) calcd for $C_{33}H_{52}NO_8$ (M^+) 590.3687, found 590.3663.

Phorbol 12-Suberate-13-acetate (8). White solid, mp 139.5 °C; $[\alpha]_D$ +17.7° (*c* 0.3, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (s, 1H), 5.68 (d, J = 4.8 Hz, 1H), 5.58 (br s, 1H), 5.40 (d, J = 10.3 Hz, 1H), 4.01 (AB, J = 13.2 Hz, 2H), 3.25 (br s, 2H), 2.56 (d, J = 19.0 Hz, 1H), 2.48 (d, J = 19.0 Hz, 1H), 2.36−2.29 (m, 4H), 2.14 (dq, J = 9.9, 6.6 Hz, 1H), 2.09 (s, 3H), 1.77−1.75 (m, 3H), 1.68−1.60 (m, 4H), 1.37−1.32 (m, 4H), 1.24 (s, 3H), 1.21 (s, 3H), 1.09 (d, J = 5.5 Hz, 1H), 0.89 (d, J = 6.2 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 208.9, 177.0, 173.5, 173.3, 160.7, 140.3, 132.8, 129.0, 78.2, 76.4, 73.7, 68.0, 65.6, 56.1, 42.9, 39.1, 38.5, 36.3, 34.5, 33.6, 28.7, 25.8, 25.0, 24.6, 23.9, 21.2, 17.0, 14.6, 10.3; IR (neat) 3400, 1712, 1629 cm^{-1}; HRMS (MALDI) calcd for $C_{30}H_{42}O_{10}Na$ (MNa^+) 585.2676, found 585.2688.

Phorbol 12-Oxalate-13-acetate (9). Clear oil; $[\alpha]_D$ +12.2° (*c* 0.5, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (s, 1H), 5.67 (d, J = 4.9 Hz, 1H), 5.54 (br s, 1H), 5.48 (d, J = 10.2 Hz, 1H), 4.01 (AB, J = 13.1 Hz, 2H), 3.29−3.22 (m, 2H), 2.51 (AB, J = 19.5 Hz, 2H), 2.27 (dq, J = 10.4, 6.4 Hz, 1H), 2.12 (s, 3H), 2.10 (s, 3H), 1.77 (d, J = 1.6 Hz, 3H), 1.20 (s, 3H), 1.14 (d, J = 5.2 Hz, 1H), 0.93 (d, J = 6.3 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 208.9, 173.6, 160.4, 140.6, 133.0, 128.5, 81.2, 78.2, 73.6, 67.9, 65.0, 56.1, 42.9, 39.0, 38.5, 36.8, 26.4, 24.0, 21.2, 16.6, 14.6, 10.3 ppm; IR (neat) 3402, 1767, 1706, 1628 cm^{-1}; LRMS (ESI) 429 ($MNa^+ - C_2O_3$).

Phorbol 12-Succinate-13-acetate (10). Clear oil; $[\alpha]_D$ +1.9° (*c* 0.13, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (s, 1H), 5.66 (d, J = 5.1 Hz, 1H), 5.59 (br s, 1H), 5.41 (d, J = 10.3 Hz, 1H), 4.01 (AB, J = 13.0 Hz, 2H), 3.24 (br s, 2H), 2.73−2.62 (m, 4H), 2.55 (d, J = 19.0 Hz, 1H), 2.48 (d, J = 19.0 Hz, 1H), 2.15 (dq, J = 10.3, 6.6 Hz, 1H), 2.08 (s, 3H), 1.76 (d, J = 1.8 Hz, 3H), 1.23 (s, 3H), 1.21 (s, 3H), 1.08 (d, J = 5.1 Hz, 1H), 0.88 (d, J = 6.6 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 208.9, 175.8, 173.6, 171.7, 160.7, 140.4, 132.7, 129.0, 78.3,

77.7, 73.6, 68.0, 65.5, 56.1, 43.0, 39.0, 38.5, 36.3, 29.2, 28.8, 25.9, 23.9, 21.2, 16.9, 14.5, 10.3 ppm; IR (neat) 3406, 1714, 1630 cm^{-1}; LRMS (ES) 505 (MH^-, 100%); HRMS (MALDI) calcd for $C_{26}H_{34}O_{10}Na$ (MNa^+) 529.2050, found 529.2026.

Phorbol 12-Adipate-13-acetate (11). Clear, pale yellow oil; $[\alpha]_D$ +7.9° (*c* 0.1, MeOH); ^1H NMR (400 MHz, $CDCl_3$) δ 7.58 (br s, 1H), 5.68 (d, J = 5.1 Hz, 1H), 5.54 (br s, 1H), 5.41 (d, J = 10.6 Hz, 1H), 4.02 (AB, J = 13.0 Hz, 2H), 3.26−3.23 (m, 2H), 2.54 (d, J = 19.8 Hz, 1H), 2.47 (d, J = 19.6 Hz, 1H), 2.41−2.35 (m, 4H), 2.18−2.11 [m, 2H containing 2.14 (dq, J = 10.6, 6.6 Hz, 1H)], 2.09 (s, 3H), 1.78−1.77 (m, 3H), 1.71−1.69 (br m, 4H), 1.24 (s, 3H), 1.20 (s, 3H), 1.09 (d, J = 5.1 Hz, 1H), 0.89 (d, J = 6.2 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.6, 172.9, 160.8, 140.3, 132.7, 128.9, 78.1, 76.5, 73.7, 68.0, 56.2, 42.9, 39.1, 38.6, 36.3, 34.3, 33.1, 25.8, 24.6, 24.1, 23.9, 21.2, 16.9, 14.6, 10.3; IR (neat) 3415, 1712 cm^{-1}; HRMS (MALDI) calcd for $C_{28}H_{38}O_{10}Na$ (MNa^+) 557.2363, found 557.2344.

Phorbol 12-Sebacate-13-acetate (12). Clear oil; $[\alpha]_D$ +3.1° (*c* 0.1, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$) δ 7.60 (s, 1H), 5.68 (d, J = 4.8 Hz, 1H), 5.59 (br s, 1H), 5.40 (d, J = 10.3 Hz, 1H), 4.02 (AB, J = 12.8 Hz, 2H), 3.30−3.20 (m, 2H), 2.57 (d, J = 19.0 Hz, 1H), 2.48 (d, J = 19.0 Hz, 1H), 2.35−2.31 (m, 4H), 2.17 (s, 1H), 2.15 (dq, J = 10.6, 6.4 Hz, 1H), 2.09 (s, 3H), 1.77 (dd, J = 2.6, 1.1 Hz, 3H), 1.66−1.58 (m, 4H), 1.67−1.58 (m, 4H), 1.37−1.28 (m, 4H), 1.24 (s, 3H), 1.21 (s, 3H), 1.09 (d, J = 5.1 Hz, 1H), 0.89 (d, J = 6.2 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.6, 173.4, 161,0, 140.4, 132.8, 129.1, 78.3, 76.6, 73.7, 68.0, 65.7, 42.9, 39.1, 38.4, 36.3, 34.6, 33.9, 31.7, 29.13, 29.06, 29.01, 28.99, 25.8, 25.2, 24.7, 23.9, 21.2, 17.0, 14.6, 10.2 ppm; IR (neat) 3410, 1712, 1629 cm^{-1}; HRMS (MALDI) calcd for $C_{32}H_{46}O_{10}Na$ (MNa^+) 613.2989, found 613.2997.

Phorbol 12-Tetradecadioate-13-acetate (13). Yellow oil; $[\alpha]_D$ +5.4° (*c* 0.25, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$) δ 7.63−7.60 (m, 1H), 5.67 (d, J = 4.4 Hz, 1H), 5.61 (br s, 1H), 5.40 (d, J = 10.3 Hz, 1H), 4.01 (AB, J = 12.8 Hz, 2H), 3.30−3.24 (m, 2H), 2.58 (d, J = 19.0 Hz, 1H), 2.48 (d, J = 19.0 Hz, 1H), 2.36−2.31 (m, 4H), 2.172 (s, 1H), 2.167 (dq, J = 10.3, 6.6 Hz, 1H), 2.09 (s, 3H), 1.77−1.76 (m, 3H), 1.67−1.57 (m, 4H), 1.35−1.22 (m, 19H), 1.21 (s, 3H), 1.08 (d, J = 5.1 Hz, 1H), 0.89 (d, J = 6.6 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 210.0, 173.93, 173.85, 161.5, 140.6, 133.0, 129.3, 78.3, 76.6, 73.7, 68.0, 65.6, 56.1, 42.8, 38.9, 38.3, 36.1, 34.6, 29.4, 29.3, 29.2, 29.13, 29.08, 29.04, 28.9, 28.8, 25.6, 25.0, 24.5, 23.7, 21.0, 16.7, 14.3, 10.0 ppm; IR (neat) 3400, 1713, 1628 cm^{-1}; HRMS (MALDI) calcd for $C_{36}H_{54}O_{10}Na$ (MNa^+) 669.3609, found 669.3594.

Phorbol 12-[(*Z*)-4-Octenedioate]-13-acetate (14). Yellow oil; $[\alpha]_D$ +10.2° (*c* 0.5, MeOH); ^1H NMR (400 MHz, $CDCl_3$) δ 7.59 (s, 1H), 5.67 (d, J = 5.1 Hz, 1H), 5.45−5.38 (m, 3H), 4.00 (AB, J = 13.0 Hz, 2H), 3.31−3.22 (m, 2H), 2.56 (d, J = 19.0 Hz, 1H), 2.48 (d, J = 19.0 Hz, 1H), 2.38 (br s, 8H), 2.16 (dq, J = 10.3, 6.6 Hz, 1H), 2.09 (s, 3H), 1.75 (d, J = 1.8 Hz, 3H), 1.24 (s, 3H), 1.20 (s, 3H), 1.08 (d, J = 5.1 Hz, 1H), 0.87 (d, J = 6.2 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 209.3, 177.6, 173.6, 172.8, 161.0, 140.4, 132.8, 129.0, 128.9, 128.8, 78.4, 76.9, 73.7, 68.0, 65.6, 56.0, 42.9, 38.9, 38.3, 36.3, 34.5, 34.0, 25.9, 23.9, 23.0, 22.7, 21.2, 16.9, 14.6, 10.3 ppm; IR (thin film) 3407, 1713, 1628 cm^{-1}; HRMS (MALDI-FTMS) calcd for $C_{30}H_{40}O_{10}Na$ (MNa^+) 583.2514, found 583.2538.

Phorbol 12-Terephthalate-13-acetate (15). Clear liquid; $[\alpha]_D$ +18.1° (*c* 0.5, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$) δ 8.17 (d, J = 8.3 Hz, 2H), 8.11 (d, J = 8.3 Hz, 2H), 7.65 (s, 1H), 5.71 (d, J = 5.4 Hz, 1H), 5.69 (d, J = 10.3 Hz, 1H), 4.04 (AB, J = 18.8 Hz, 2H), 3.35 (t, J = 4.9 Hz, 1H), 3.31 (t, J = 2.4 Hz, 1H), 2.59 (d, J = 18.8 Hz, 1H), 2.53 (d, J = 18.8 Hz, 1H), 2.36 (dq, J = 10.3, 6.3 Hz, 1H), 2.13 (s, 3H), 2.11 (s, 1H), 1.78−1.76 (m, 3H), 1.38 (s, 3H), 1.20 (s, 3H), 1.16 (d, J = 4.9 Hz, 1H), 0.94 (d, J = 6.3 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 210.2, 174.0, 169.6, 165.7, 161.7, 140.7, 134.4, 133.1, 130.3, 129.8, 129.2, 78.40, 78.37, 73.8, 68.0, 65.6, 56.2, 43.1, 39.0, 38.3, 36.5, 26.0, 23.7, 21.0, 17.0, 14.4, 10.0 ppm; IR (neat) 3396, 1716, 1708, 1626 cm^{-1}; LRMS (ES-neg ion) 553 (M−H, 100); HRMS (MALDI) calcd for $C_{30}H_{34}O_{10}Na$ (MNa^+) 577.2044, found 577.2017.

Phorbol 12,13-Diterephthalate (16). Viscous yellow oil; $[\alpha]_D$ +3.6° (*c* 0.3, MeOH); ^1H NMR (400 MHz, CD_3OD) δ 8.14−8.08 (m, 8H), 7.62 (s, 1H), 5.86 (d, J = 10.3 Hz, 1H), 5.71 (d, J = 4.4 Hz, 1H), 3.97 (AB, J = 13.0 Hz, 2H), 3.46 (t, J = 5.1 Hz, 1H), 3.25 (t, J = 2.7 Hz, 1H), 2.59 (d, J = 19.0 Hz, 1H), 2.53 (d, J = 19.0 Hz, 1H), 2.48 (dq, J = 10.3, 6.3 Hz, 1H), 1.75 (d, J = 1.5 Hz, 3H), 1.48 (s, 3H), 1.40 (d, J = 5.4 Hz, 1H), 1.31 (s, 3H), 0.98 (d, J = 6.3 Hz, 3H) ppm; ^{13}C NMR (100 MHz, CD_3OD) δ 210.0, 177.8, 169.0, 168.6, 167.0, 160.2, 142.8, 134.6, 134.5, 134.2, 130.70, 130.66, 130.5, 129.0, 79.9, 79.8, 74.7, 68.1, 68.0, 57.5, 44.8, 40.2, 38.5, 37.6, 28.4, 24.3, 17.8, 15.1, 10.3 ppm; IR (neat) 3405, 1785, 1706 cm^{-1}; HRMS calcd for $C_{36}H_{34}O_{12}Na$ [$(M - 2H^+)Na^+$] 681.1953, found 681.1926.

Phorbol 12,13-Disuccinate (17). Clear, yellow oil; $[\alpha]_D$ +22.0° (*c* 0.2, MeOH); ^1H NMR (400 MHz, CD_3OD) δ 7.56 (s, 1H), 5.62 (d, J = 4.9 Hz, 1H), 5.44 (d, J = 10.7 Hz, 1H), 3.94 (AB, J = 13.2 Hz, 2H), 3.30 (t, J = 5.4 Hz, 1H), 3.17−3.14 (m, 1H), 2.62−2.60 (m, 8H), 2.54 (d, J = 19.5 Hz, 1H), 2.48 (d, J = 19.0 Hz, 1H), 2.23 (dq, J = 10.5, 6.6 Hz, 1H), 2.03 (s, 1H), 1.74 (dd, J = 2.9, 1.0 Hz, 3H), 1.26 (s, 3H), 1.22 (s, 3H), 1.15 (d, J = 5.4 Hz, 1H), 0.90 (d, J = 6.8 Hz, 3H) ppm; ^{13}C NMR (100 MHz, CD_3OD) δ 210.6, 177.0, 175.9, 174.5, 160.7, 142.9, 134.7, 129.4, 79.9, 78.8, 74.7, 68.0, 67.2, 57.3, 44.3, 39.9, 38.4, 36.9, 30.4, 30.3, 29.9, 29.6, 27.5, 23.9, 17.3, 14.7, 10.1 ppm; IR (neat) 3405, 1715 cm^{-1}; HRMS (MALDI) calcd for $C_{28}H_{36}O_{12}$-Na (MNa^+) 587.2099, found 587.2107.

Phorbol 12-Acetate-13-succinate (18). Clear, viscous liquid; $[\alpha]_D$ +7.7° (*c* 0.3, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$) δ 7.55 (s, 1H), 5.80 (br s, 1H), 5.64 (d, J = 4.4 Hz, 1H), 5.33 (d, J = 10.3 Hz, 1H), 3.97 (AB, J = 13.0 Hz, 2H), 3.26−3.22 (m, 2H), 2.84−2.75 (m, 1H), 2.70−2.60 (m, 1H), 2.59−2.48 (m, 4H), 2.19−2.14 [m, 2H containing 2.16 (s, 1H)], 2.09 (br s, 1H), 2.06 (s, 3H), 1.73 (d, J = 1.5 Hz, 3H), 1.23 (s, 3H), 1.22 (s, 3H), 1.07 (d, J = 4.9 Hz, 1H), 0.86 (d, J = 6.3 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 209.7, 176.0, 175.2, 170.9, 161.0, 140.7, 132.9, 128.4, 78.6, 76.9, 73.7, 67.3, 66.0, 55.9, 42.8, 38.8, 38.2, 36.1, 29.1, 28.6, 26.2, 23.8, 21.2, 17.0, 14.6, 10.3 ppm; IR (neat) 3401, 1725, 1629 cm^{-1}; HRMS (MALDI) calcd for $C_{26}H_{34}O_{10}Na$ (MNa^+) 529.2044, found 529.2026.

Phorbol 12-Acetate-13-terephthlate (19). White powder, mp 189−190 °C; $[\alpha]_D$ +17.5° (*c* 0.1, CH_2Cl_2); ^1H NMR (400 MHz, acetone-d_6) δ 8.18−8.08 (m, 4H), 7.56 (s, 1H), 5.68 (d, J = 4.4 Hz, 1H), 5.57 (d, J = 10.3 Hz, 1H), 5.33 (br s, 1H), 3.96 (AB, J = 13.0 Hz, 2H), 3.36 (t, J = 5.0 Hz, 1H), 3.22 (t, J = 2.2 Hz, 1H), 2.57 (d, J = 19.4 Hz, 1H), 2.51 (d, J = 19.4 Hz, 1H), 2.32 (dq, J = 10.3, 6.6 Hz, 1H), 2.14 (s, 1H), 2.08 (s, 3H), 1.71 (d, J = 1.5 Hz, 3H), 1.40 (d, J = 5.1 Hz, 1H), 1.37 (s, 3H), 1.35 (s, 3H), 1.19 (s, 1H), 0.93 (d, J = 6.6 Hz, 3H) ppm; ^{13}C NMR (100 MHz, acetone-d_6) δ 208.8, 171.8, 169.0, 160.0, 143.4, 134.1, 131.2 (br), 129.0, 79.5, 78.4, 75.2, 68.5, 68.3, 57.8, 44.9, 40.7, 39.2, 37.7, 28.1, 25.0, 21.8, 18.2, 15.7, 11.2 ppm; IR (neat) 3412, 1729, 1703, 1628 cm^{-1}; HRMS (MALDI) calcd for $C_{30}H_{34}O_{10}Na$ (MNa^+) 577.2044, found 577.2070.

Phorbol 12-Myristate-13-succinate (20). Colorless oil; $[\alpha]_D$ +6.2° (*c* 0.1, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$) δ 7.63−7.60 (m, 1H), 5.67 (d, J = 4.4 Hz, 1H), 5.61 (br s, 1H), 5.40 (d, J = 10.3 Hz, 1H), 4.01 (AB, J = 12.8 Hz, 2H), 3.30−3.24 (m, 2H), 2.58 (d, J = 19.0 Hz, 1H), 2.48 (d, J = 19.0 Hz, 1H), 2.36−2.31 (m, 4H), 2.172 (s, 1H), 2.167 (dq, J = 10.3, 6.6 Hz, 1H), 2.09 (s, 3H), 1.77−1.76 (m, 3H), 1.67−1.57 (m, 4H), 1.35−1.22 (m, 19H), 1.21 (s, 3H), 1.08 (d, J = 5.1 Hz, 1H), 0.89 (d, J = 6.6 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 210.0, 173.93, 173.85, 161.5, 140.6, 133.0, 129.3, 78.3, 76.6, 73.7, 68.0, 65.6, 56.1, 42.8, 38.9, 38.3, 36.1, 34.6, 29.4, 29.3, 29.2, 29.13, 29.08, 29.04, 28.9, 28.8, 25.6, 25.0, 24.5, 23.7, 21.0, 16.7, 14.3, 10.0 ppm; IR (neat) 3400, 1713, 1628 cm^{-1}; HRMS (MALDI) calcd for $C_{36}H_{54}O_{10}Na$ (MNa^+) 669.3609, found 669.3594.

Phorbol 12-Acetate-13-tetradecanedioate (21). Yellow oil; $[\alpha]_D$ +2.4° (*c* 0.1, CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$) δ

7.59 (s, 1H), 5.82 (br s, 1H), 5.68 (d, J = 5.0 Hz, 1H), 5.38 (d, J = 10.3 Hz, 1H), 4.01 (AB, J = 12.3 Hz, 2H), 3.28 (t, J = 2.6 Hz, 1H), 3.25 (t, J = 5.3 Hz, 1H), 2.52 (br s, 2H), 2.34−2.26 (m, 4H), 2.15 (dq, J = 10.3, 6.2 Hz, 1H), 2.07 (s, 3H), 1.77 (d, J = 1.5 Hz, 3H), 1.67−1.58 (m, 4H), 1.42−1.20 [m, 22H, containing 1.24 (s, 3H), 1.22 (s, 3H)], 1.05 (d, J = 5.3 Hz, 1H), 0.89 (d, J = 6.4 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 209.5, 176.6, 171.1, 161.1, 140.6, 133.0, 129.3, 78.4, 77.1, 73.7, 67.9, 65.3, 56.0, 42.9, 38.9, 38.4, 36.3, 34.2, 29.6, 29.2, 29.1, 29.0, 28.9, 25.8, 24.7, 24.4, 23.8, 20.9, 16.7, 14.3, 10.0 ppm; IR (thin film) 3401, 1713 cm^{-1}; HRMS (MALDI-FTMS) calcd for $C_{36}H_{54}O_{10}Na$ (MNa^+) 669.3609, found 669.3621.

Protein Kinase C βII. Protein kinase C βII from the baculovirus expression system was purified as described previously[49] and stored at −20 °C in 10 mM Tris buffer, pH 7.5 (4 °C), 0.5 mM EDTA, 0.5 mM EGTA, 0.5 mM dithiothreitol, and 50% (v/v) glycerol.

Sucrose-Loaded Vesicles. A lipid mixture composed of 30 mol % of PS and 70 mol % of POPC in chloroform/methanol was dried under a stream of dry nitrogen and subsequently concentrated in vacuo (0.1 mmHg). Lipids were then resuspended in a sucrose buffer (pH 7.5) containing 0.170 M sucrose, 5 mM $MgCl_2$, and 20 mM HEPES. Aliquots (0.5 mL) of 5 mM lipid were subjected to 5 freeze−pump−thaw cycles in liquid nitrogen, followed by 21 rounds of extrusion through two stacked 100-nm polycarbonate filters in a microextruder to form large unilamellar vesicles. Both extrusion of vesicles and subsequent incorporation of phorbol esters into them (see below) was accomplished at 20 °C. The vesicles were suspended in 100 mM KCl, 20 mM HEPES (pH 7.5) and centrifuged at 100 000 × g for 30 min. The pelleted sucrose-loaded vesicles were resuspended in buffer to achieve a concentration of 1.0 mM lipid (all phospholipid concentrations in stock solutions were determined by phosphate analysis). At later stages of experiments, the lipid concentration was calculated from the amount of [^{3}H]DPPC that was routinely included in trace quantities in lipid mixtures.[50]

Incorporation of Phorbol Esters into Vesicles. Stock solutions of all phorbol esters were prepared as DMSO solutions degassed by 3 freeze−pump−thaw cycles under vacuum (0.1 mmHg) and stored at −22 °C in the dark.[33] Appropriate aliquots of concentrated PMA or the synthetic phorbol ester in dimethyl sulfoxide were injected into vigorously vortexed suspensions of large unilamellar vesicles. Subsequently, vesicles were incubated for 30 min at 20 °C with occasional gentle vortexing. The partitioning of PMA into vesicles was complete (>98%) as assessed by centrifugation of sucrose-loaded vesicles and analysis of bound [20-^{3}H]PMA. Appropriate aliquots of suspensions of vesicles containing the phorbol esters were diluted several-fold in binding assay samples. The final content of DMSO did not exceed 0.5% (v/v) and did not change the association of the enzyme with vesicles to any appreciable degree.

Protein Kinase C-βII Recruitment Assay. The sucrose-loaded vesicle assay was based on the procedure of Rebecchi et al.[51] adapted for protein kinase C as previously described,[52] except final volumes were 525 μL. Membrane-bound enzyme was separated by centrifugation at 100 000 × g for 30 min at 25 °C to ensure ≥95% sedimentation of large unilamellar vesicles. Both the supernatant and the pellet were assayed under identical conditions for kinase activity toward protamine sulfate, as described previously.[53] This substrate is phosphorylated by PKC in a cofactor (eg. lipids, PMA, Ca^{2+})-independent manner. Therefore, the ratio of activity obtained in the supernatant and pellet will not be affected by phorbol analogues. To further ensure this, equal amounts of vesicles/analogues are added back to the supernatant to provide identical conditions. The phosphorylation reaction was initiated by the addition of 15 μL of 0.5 mM [γ-^{32}P]ATP (75 μCi/mL), 25 mM $MgCl_2$, 20 mM HEPES (pH 7.5), and 0.5 mg mL^{-1} protamine sulfate to a 60 μL reaction volume of supernatant or pellet fraction. Phosphorylation was carried out for 10 min at 25 °C and quenched with 25 μL of 0.1 M ATP and 0.1 M EDTA (pH 8.0). Samples (85 μL) were spotted on P-81 ion-exchange paper and subsequently washed with 0.4% (v/v) phosphoric acid for 20 min. Radioactivity associated with papers was determined by liquid scintillation counting in Biosafe II. The vesicle-associated kinase activity, A_v, was calculated according to eq 1, where A_b and A_t are the measure activities of the bottom and top fractions, respectively:

$$A_v=[\beta A_b + (\beta - 1)A_t]/[\alpha + \beta - 1] \quad (1)$$

The fraction of sedimented vesicles, α, was calculated from the distribution of [^{3}H]-labeled PC, which was included in trace amounts in all lipid mixtures. The fraction of kinase activity found in the supernatant in the absence of lipid, β, was equal—within the limits of experimental error—to the value expected for a nonsedimenting protein (i.e. 0.73 under the experimental conditions used). All experiments were performed in a standard solution composed of 100 mM KCl, 5 mM $MgCl_2$, 20 mM HEPES (pH 7.5), 200 μM Ca^{2+}, and 0.3 mg mL^{-1} of BSA. Concentrations of additions that varied depending on experiment are given with appropriate results. The fraction of membrane-bound enzyme was calculated as $[A_v/(A_b + A_t)]$. The distribution of PKC between the supernatant and pellet measured by the sucrose-loaded vesicle assay is the same as that obtained with use of a Western Blot or silver stain to physically determine the distribution of the protein.

The PKC-catalyzed phosphorylation of the peptide ac-FKKSFKL-amide was determined in the presence of PMA or analogue. The assay consisted of 100 mM KCl, 5 mM $MgCl_2$, 20 mM HEPES (pH 7.5), 200 μM Ca^{2+}, 0.3 mg/mL of BSA, 0.2 mg/mL of peptide, and 0.1 mM ATP. The reaction was carried out for 6 min at 25 °C. Samples were treated as above for all remaining steps. Activity is reported as a percentage of that obtained with 1 μM PMA.

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Supporting Information Available: Complete experimental procedures and ^{1}H and ^{13}C NMR spectra for compounds **3**−**21** and their precursors. This material is available free of charge via the Internet at http://pubs.acs.org.

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