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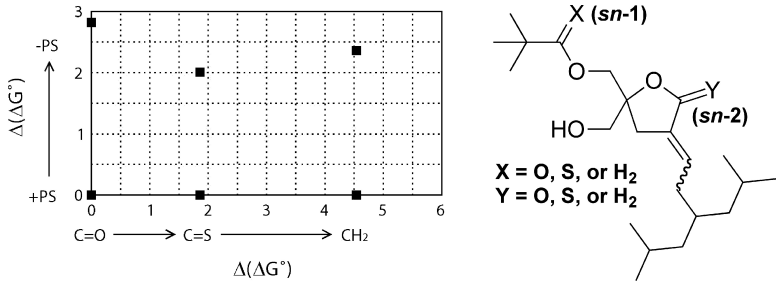

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Conformationally Constrained Analogues of Diacylglycerol (DAG). 25. Exploration of the *sn*-1 and *sn*-2 Carbonyl Functionality Reveals the Essential Role of the *sn*-1 Carbonyl at the Lipid Interface in the Binding of DAG-Lactones to Protein Kinase C

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Diacylglycerol (DAG) lactones with altered functionality (C=O → CH_2 or C=O → C=S) at the *sn*-1 and *sn*-2 carbonyl pharmacophores were synthesized and used as probes to dissect the individual role of each carbonyl in the binding to protein kinase C (PKC). The results suggest that the hydrated *sn*-1 carbonyl is engaged in very strong hydrogen-bonding interactions with the charged lipid headgroups and organized water molecules at the lipid interface. Conversely, the *sn*-2 carbonyl has a more modest contribution to the binding process as a result of its involvement with the receptor (C1 domain) via conventional hydrogen bonding to the protein. The parent DAG-lactones, ***E*-6** and ***Z*-7**, were designed to bind exclusively in the *sn*-2 binding mode to ensure the correct orientation and disposition of pharmacophores at the binding site.

Introduction

The lipophilic second messenger, *sn*-1,2-diacylglycerol (*sn*-1,2-DAG), plays a prominent role in cellular signal transduction.[1−3] Generated through both G-protein-coupled and tyrosine kinase activated isoforms of phospholipase C, as well as indirectly by phospholipase D, DAG binds to the C1 domains (C1a or C1b) of protein kinase C (PKC) isozymes and other non-kinase protein targets activating their downstream pathways.[4,5] The importance of these pathways in cellular responses, including proliferation, differentiation, gene expression, and tumor promotion, has been well documented in the literature in studies with the phorbol esters, which function as potent and metabolically stable DAG surrogates.[6]

Both conventional (α, β1, β2, and γ) and novel (δ, ϵ, η, and θ) PKC isozymes are thought to be activated as a result of association of the cytosolic enzyme with membranes containing acid phospholipids.[7,8] This association is strongly facilitated by the liberation of DAG which causes the transient translocation of PKC to the inner leaflet of the cellular membrane.[9−11]

To accelerate our understanding of the structure−activity analysis of ligand and C1 domain interactions, we have developed a chemically accessible template in the form of a rigid lactone that contains a conformationally constrained glycerol backbone.[12] The resulting DAG-lactones seem to overcome part of the entropic penalty associated with the binding of DAG, and nanomolar binding affinities in the range normally observed for the phorbol esters have been achieved in vitro.[12]

Ever since the X-ray structure of the binary complex of phorbol 13-*O*-acetate bound to the C1b domain of PKCδ was solved, the role of the C9 OH pharmacophore in phorbol has remained elusive.[13] This critical pharmacophore does not appear to be engaged at all with the receptor, but instead, it forms an intramolecular hydrogen bond with the C13 carbonyl ester of phorbol itself. Although it is possible that such an intramolecular hydrogen bond could be biologically relevant, the more likely explanation is that its formation is improperly facilitated by the absence of a lipid bilayer in the crystal structure. Indeed, recent molecular modeling studies performed by Miskovsky et al.[14] on a binary complex of phorbol myristate (PMA) and a dipalmitoyl phosphatidylcholine (DPPC) bilayer (PMA-DPPC) or on the more relevant ternary C1b−PMA-DPPC complex illustrate convincingly the important role of the furtive C9 OH by uncovering strong hydrogen-bonding interactions of this OH directly to the phosphate group or with the water molecules surrounding the headgroups of DPPC.

In a similar manner, our modeling studies on binary complexes involving the C1 domain and DAG-lactones have also shown that for either one of the two binding modes identified (*sn*-1 or *sn*-2)[15] there is an orphan carbonyl pharmacophore whose role we propose is equivalent to that of the C9 OH of phorbol. These two apparently comparable binding modes for the DAG-lactones are able to form identical networks of hydrogen bonds with amino acids Thr242, Leu251, and Gly253, as was observed with phorbol 13-*O*-acetate.[13] The *sn*-1 binding mode is defined as that in which the *sn*-1

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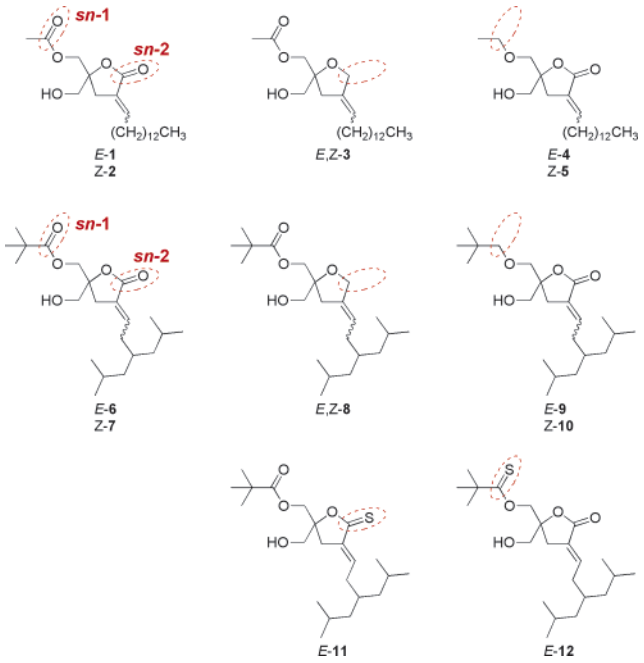
Chart 1. Structures of Compounds **1**−**12**



carbonyl is hydrogen-bonded to the C1 domain, and for the alternative *sn*-2 binding mode, it is the *sn*-2 carbonyl that appears directly engaged in hydrogen bonding to the protein.

In a preliminary study designed to determine the importance of these nonequivalent carbonyl moieties, we synthesized compounds **3**, **4**, and **5** with the intent to dissect the importance of each individual carbonyl relative to the parent DAG-lactones **1** (*E*-isomer) and **2** (*Z*-isomer) (Chart 1).[16] Compounds **4** and **5** were individually assayed as *E*- and *Z*-isomers, respectively, whereas compound **3** was evaluated as a mixture of the two geometric isomers. Because all the compounds showed an indistinct ∼100-fold decrease in binding affinity relative to the parent compounds, it was impossible to assess the independent role of each carbonyl (*sn*-1 or *sn*-2) toward binding, and the only conclusion that could be drawn was that the presence of both groups was essential. However, in ensuing studies we were able to design DAG-lactones, such as **6** (*E*-isomer) and **7** (*Z*-isomer), that showed an unequivocal preference for the *sn*-2 binding mode due to the large branched alkyl chain being positioned adjacent to the lactone carbonyl (Figure 1).[15,17] We proposed that utilizing this new and more potent DAG-lactone template could improve our chances of diagnosing the different roles played by each carbonyl in the binding process. Indeed, the difference in binding affinity between DAG-lactone **1** versus **6** and between **2** versus **7** was respectively17- to12-fold higher, suggesting that compounds **6** and **7** were better candidates for the study.

Having the role of the lactone (*sn*-2) carbonyl defined as bound to the C1 domain (*sn*-2 binding mode, Figure 1), the hypothesis was that in the "real life" ternary complex the apparently orphan *sn*-1 carbonyl pharmacophore (as it appears in the binary complex) would be directed to the membrane interface where it would bind to either organized water molecules or the lipid headgroups. If our assumption were correct, elimination of either of these carbonyls, represented by compounds **8** (*E*,*Z*-isomers), **9** (*E*-isomer), and **10** (*Z*-isomer), or replacement by a thiocarbonyl moiety, as in compounds **11** (*E*-isomer) and **12** (*E*-isomer), would affect the binding affinities of the ligands as a function of the binding environment of each carbonyl.

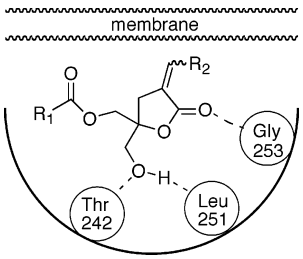
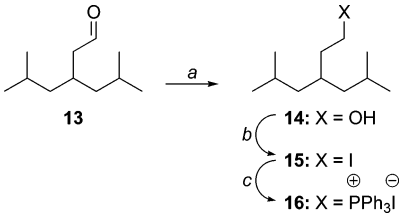


Figure 1. Schematic representation of the *sn*-2 binding mode of DAG-lactones.

Scheme 1[a]



[a] Reagents and conditions: (a) $LiAlH_4$/THF, 0 °C; (b) PPh_3, imidazole, I_2, THF, room temp; (c) PPh_3, $PhCH_3$, reflux.

The changes in binding affinities that were measured confirmed the above hypothesis and suggest that the two carbonyls indeed reside in different environments with the *sn*-1 carbonyl engaged in strong polar interactions at the interface and capable of playing a role similar to that proposed for the C9 OH of phorbol.[14]

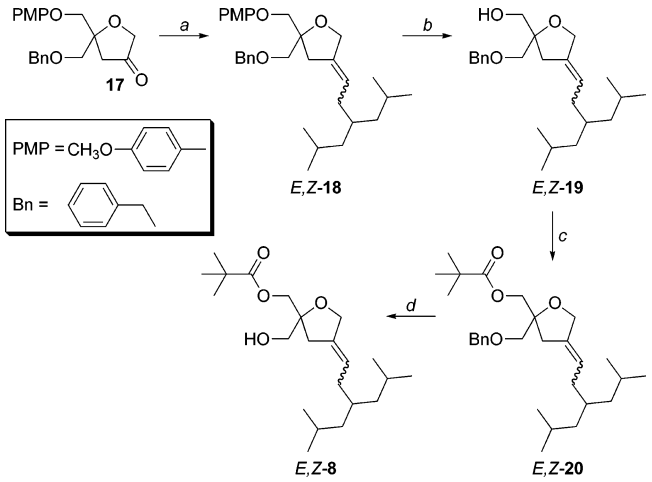
Chemistry

The two critical branched chain components for this project were the previously used aldehyde **13**[17−19] and 5-methyl-3-(2-methylpropyl)hexyl]triphenylphosphonium iodide (**16**) (Scheme 1). The latter compound was obtained from **13** via lithium aluminum hydride reduction to the alcohol (**14**), iodination with Ph_3P/imidazole/I_2 to give 4-(2-iodoethyl)-2,6-dimethylheptane (**15**), and final treatment with triphenylphosphine to afford the desired phosphonium salt (**16**) as a white solid.

The DAG-lactones missing the *sn*-2 carbonyl were synthesized via Wittig reaction with the known 5-[(4-methoxyphenoxy)methyl]-5-[(phenylmethoxy)methyl]-2,4,5-trihydrofuran-3-one (**17**)[20] and the corresponding ylid generated from **16** with *n*-butyllithium (Scheme 2). Compound **18** was obtained as an inseparable mixture of geometric isomers. Removal of the *p*-methoxyphenyl (PMP) group with ammonium cerium(IV) nitrate afforded monoalcohol **19**, and acylation with pivaloyl chloride followed by deprotection of the benzyl ether with BCl_3 at −78 °C provided the target compound **8** as an inseparable mixture of geometric isomers. Judging from the integration of the pivaloyl methyl [C(O)C$(CH_3)_3$] signal, the ratio of isomers was estimated to be 14:1, but the exact geometry of the double bond of the predominant isomer could not be determined.

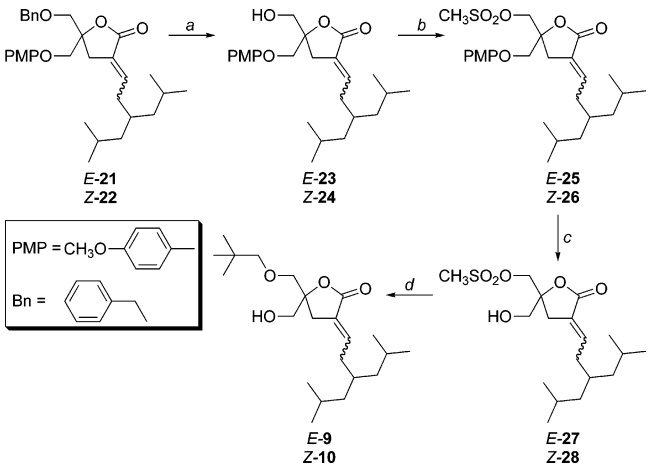
The DAG-lactone targets devoid of the *sn*-1 carbonyl **(*E*-9** and ***Z*-10)** were synthesized, respectively, from the isomers *E*-**21** and *Z*-**22**,[19] which were prepared according

Scheme 2[a]



[a] Reagents and conditions: (a) **16**, *n*-BuLi/THF, 0 °C → reflux; (b) $(NH_4)_2Ce(NO_3)_6/CH_3CN/H_2O$, 0 °C; (c) $(CH_3)_3C(O)Cl$, Et_3N/CH_2Cl_2 (0 °C); (d) BCl_3, CH_2Cl_2, −78 °C.

Scheme 3[a]

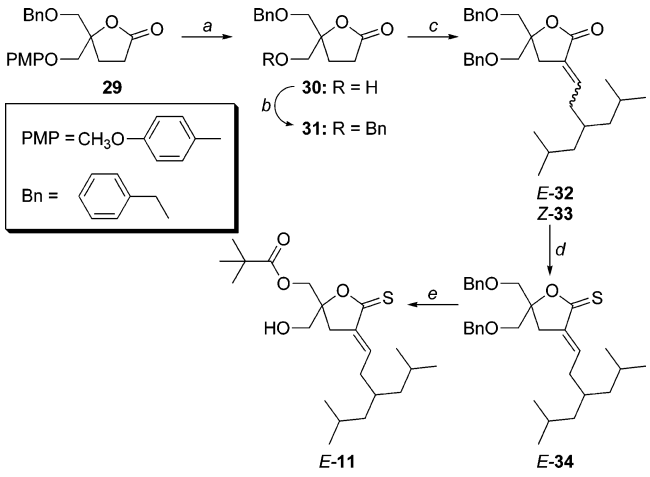


[a] Reagents and conditions: (a) BCl_3, CH_2Cl_2, −78 °C; (b) CH_3SO_2Cl, Et_3N, CH_2Cl_2, 0 °C; (c) $(NH_4)_2Ce(NO_3)_6/CH_3CN/H_2O$, 0 °C; (d) $(CH_3)_3CH_2OH$, Et_3N, DMF, 0 °C.

to our published method (Scheme 3). Removal of the benzyl ether with BCl_3 at −78 °C gave the corresponding monoalcohols *E*-**23** and *Z*-**24**, which were subsequently converted to the corresponding methylsulfonate esters *E*-**25** and *Z*-**26**. Deprotection of the *p*-methoxyphenyl ether with ammonium cerium(IV) nitrate provided monoalcohols *E*-**27** and *Z*-**28**, and displacement of the mesylate ester with neopentyl alcohol gave the desired targets *E*-**9** and *Z*-**10**.

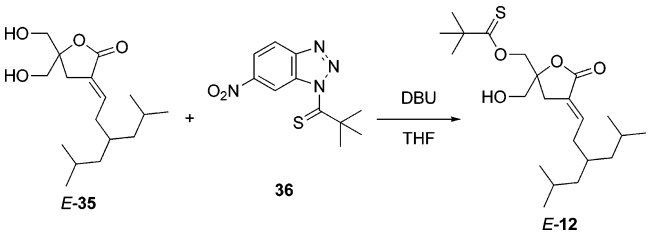
The strategy for the synthesis of the thiolactone target *E*-**11** started with the known lactone, 5-[(4-methoxyphenoxy)methyl]-5-[(phenylmethoxy)methyl]-3,4,5-trihydrofuran-2-one (**29**),[19] which was converted to **31** in two easy steps (Scheme 4). Condensation of **31** with aldehyde **13**, followed by in situ conversion of the intermediate aldol adduct to the olefin by the presence of triethylamine, DBU, and methanesulfonyl chloride, afforded the individual geometric isomers *E*-**32** and *Z*-**33**, which were individually separated by column chromatography. Consistent with previously synthesized DAG-lactones, the vinyl proton of the *Z*-isomer displayed a characteristic multiplet at δ 6.12−6.18 in its ^{1}H NMR spectrum, while the corresponding signal

Scheme 4[a]



[a] Reagents and conditions: (a) $(NH_4)_2Ce(NO_3)_6$, CH_3CN-H_2O (0 °C); (b) $PhCH_2Br$, NaH, DMF, room temp; (c) (i) **13**, $[(CH_3)_3Si]_2$-NLi/THF, −78 °C; (ii) CH_3SO_2Cl, Et_3N, DBU, CH_2Cl_2, room temp; (d) Lawesson's reagent, $PhCH_3$, reflux; (e) (i) BCl_3, CH_2Cl_2, −78 °C; (ii) $(CH_3)_3COCl$, Et_3N, CH_2Cl_2, 0 °C.

Scheme 5



of the *E*-isomer appeared more downfield at δ 6.72−6.77. In the following step, regardless of the geometry of the isomer selected as the starting material, the reaction with Lawesson's reagent at 110 °C in toluene generated exclusively the thiolactone *E*-isomer (*E*-**34**). This assignment is based on the fact that the *E*-isomers are thermodynamically more stable than the *Z*-isomers and also because of the appearance of the vinyl proton signal at δ 7.05−7.10 is even further downfield compared to the lactone *E*-isomer (*E*-**32**). The diol generated from *E*-**34** after treatment with BCl_3 at −78 °C was immediately acylated with 1 equiv of pivaloyl chloride to afford the target compound *E*-**11** as a yellowish oil.

DAG-lactone *E*-**12**, with the thiocarbonyl group at the *sn*-1 position, was accessible from diol *E*-**35**, which was easily obtained from *E*-**32** (Scheme 5). According to the method of Salaby and Rapoport,[21] a solution of *E*-**35** was treated with 2,2-dimethyl-1-(6-nitrobenzotriazolyl)propane-1-thione (**36**) in the presence of DBU to give the desired target *E*-**12** as a yellowish oil.

A few remarks about the chemistry are in order. In these DAG-lactones there is a single asymmetric carbon. However, because DAG-lactones *E*-**6** and *Z*-**7** possess a large branch chain at the *sn*-2 position, they are extremely potent PKC ligands with K_i values in the nanomolar range, and the difference between a pure enantiomer and its racemate is very small (∼2 versus 4 nM).[22] Therefore, for this investigation only racemic mixtures were synthesized. The thermodynamically more stable isomer is usually the *E*-isomer, which is normally obtained in a higher ratio. Although some small differences in affinity have been detected between

Table 1. Inhibition of [^{3}H]PDBU Binding to PKCα and to C1bδ by DAG-Lactones with Modified *sn*-2 or *sn*-1 Carbonyl Functions

compd	structural change	K_i(PKC) (nM)	K_i(C1bδ) (nM)
***E*-6**		3.25 ± 0.15	0.90 ± 0.07
***Z*-7**		2.90 ± 0.35	1.16 ± 0.07
***E,Z*-8**	*sn*-2 (CH_2)	622 ± 47	219 ± 23
***E*-9**	*sn*-1 (CH_2)	14290 ± 840	1890 ± 190
***Z*-10**	*sn*-1 (CH_2)	5874 ± 20	2140 ± 130
***E*-11**	*sn*-2 (C=S)	23.7 ± 2.2	3.13 ± 0.14
***E*-12**	*sn*-1 (C=S)	113 ± 12	20.6 ± 1.1

geometric isomers, these also tend to be small (≤2-fold).[22] Because the thiolactone target ***E*-11** could only be obtained as the *E*-isomer, we concentrated our synthetic effort in obtaining the complete *E*-isomer series for all the target compounds in order to perform a comparative SAR study (vide infra). The synthetic schemes that are described here are quite adaptable to the preference of the individual chemist for a particular protecting group or method of deprotection; thus, there are several alternatives to reach the target compounds besides the ones shown in the schemes.

A final point of interest is the stability of the thiolactone ring in compound ***E*-11**. When this compound was initially synthesized, the mass spectrum showed the corresponding MH^+ ion peak at 399 for the thiolactone and a weak peak at 383 for the protonated lactone. Two months later, while standing at room temperature, the intensity of the peaks was reversed showing a ratio of products overwhelmingly in favor of the lactone. Conversion of the thiolactone ring to lactone could be catalyzed by trace amounts of acid and moisture. Under the same conditions, however, compound ***E*-12** remained stable. On the basis of these observations, the biological assay of these samples was performed with freshly synthesized materials.

Biological Results and Discussion

The PKC binding affinity for all the ligands is expressed as K_i, which reflects the ability of the compounds to displace [20-^{3}H]phorbol 12,13-dibutyrate (PDBU) from the enzyme or isolated C1 domain in a competition assay.[23]

As shown in Table 1, for the set of compounds where the carbonyl at either the *sn*-1 or *sn*-2 position was eliminated (C=O → CH_2), removal of the *sn*-1 carbonyl from the parent DAG-lactones ***E*-6** and ***Z*-7** precipitated a more severe drop in binding affinity than the removal of the *sn*-2 carbonyl. This effect was most pronounced in the case of compound ***E*-9** (Table 1). Unfortunately, the compound devoid of the *sn*-2 carbonyl (**8**) could not be separated into its geometric isomers. The removal of these carbonyl groups follows a similar trend for either the intact isozyme α or the isolated C1bδ domain. For the set of compounds where the carbonyl function is replaced with a thiocarbonyl (C=O → C=S), the drop in binding affinity follows a similar trend as for the removal of the entire function, but the effects are less dramatic. The thiocarbonyl compounds were obtained only as *E*-isomers (vide supra).

To study the effects of the lipid environment, we also compared the changes in binding affinity in the presence and absence of phosphatidylserine (PS).[24] To make a better comparison between the effects of removing the carbonyls or replacing them with the thiocarbonyl function in either the presence or absence of PS, we decided to analyze these effects on the smaller C1bδ domain using only the *E*-isomers: compounds ***E*-6**, ***E,Z*-8**, and ***E*-9** (C=O → CH_2) and compounds ***E*-6**, ***E*-11**, and ***E*-12** (C=O → C=S) (Table 2). The isolated C1bδ domain has been shown to translocate to cellular membranes in response to DAG or phorbol signaling, suggesting that its ligand binding and membrane interactions are similar in isolation and in the full-length protein. Testing the effects of removing PS on the isolated C1bδ domain eliminates the confounding factor of the C2 domain, which also interacts with charged lipid membranes.

Table 2. Inhibition of [^{3}H]PDBU Binding to C1bδ by DAG-Lactones with Modified *sn*-2 or *sn*-1 Carbonyl Functions in the Presence or Absence of Phosphatidylserine (PS)

compd	structural change	K_i(C1bδ, +PS) (nM)	K_i(C1bδ, −PS) (nM)
***E*-6**		0.90 ± 0.07	103 ± 16
***E,Z*-8**	*sn*-2 (CH_2)	219 ± 23	6620 ± 640
***E*-9**	*sn*-1 (CH_2)	1890 ± 190	101000 ± 10000
***E*-11**	*sn*-2 (C=S)	3.13 ± 0.14	173.4 ± 4.8
***E*-12**	*sn*-1 (C=S)	20.6 ± 1.1	606 ± 50

To understand the observed changes in Table 2 in thermodynamic terms, we considered the K_i value to be equivalent to the dissociation constant (K_d) for the enzyme−ligand complex. In that case, the free energy for the binding of the parent DAG-lactone (***E*-6**) can be expressed as

$$\Delta G^\circ = -RT \ln \frac{1}{K_{d(A)}}$$

This value corresponds to a reference state A, which could be compared to other states (B) representing structural or environmental changes in the following manner:

$$\Delta(\Delta G^\circ) = RT \ln \frac{K_{d(B)}}{K_{d(A)}}$$

Using the value of R as 0.00198 kcal mol^{-1} K^{-1} and assuming room-temperature conditions (300 K), we would have

$$\Delta(\Delta G^\circ) = 0.594 \ln \frac{K_{d(B)}}{K_{d(A)}}$$

The $\Delta(\Delta G^\circ)$ values in a +PS environment will reflect the changes caused by the structural modifications in that medium, whereas $\Delta(\Delta G^\circ)$ values in a −PS environment will represent the changes caused by the removal of the phosopholipid for each of the molecular alterations as shown in Tables 3 and 4 for the *sn*-1 and *sn*-2 carbonyls. All the K_i values for these calculations were taken from Table 2.

When these results are plotted in a graph, the analysis of the data is enormously simplified (Figure 2). As can be seen along the *x*-axis in parts A and B of Figure 2, complete removal of either carbonyl results in poorer binding; however, the effect is much more pronounced when the *sn*-1 carbonyl is eliminated.

Table 3. $\Delta(\Delta G°)$ Variations Caused by Structural Changes of the *sn*-1 Carbonyl in DAG-Lactones in the Presence or Absence of Phosphatidyl Serine (PS)

+PS			−PS	
$K_{d(B)}/K_{d(A)}$	$\Delta(\Delta G°)$ (kcal/mol)		$K_{d(B)}/K_{d(A)}$	$\Delta(\Delta G°)$ (kcal/mol)
↓ K_d(CO)/K_d(CO)	0	→	K_d(CO)/K_d(CO)	2.82
↓ K_d(CS)/K_d(CO)	1.86	→	K_d(CS)/K_d(CS)	2.01
↓ K_d(CH_2)/K_d(CO)	4.54	→	K_d(CH_2)/K_d(CH_2)	2.36

Table 4. $\Delta(\Delta G°)$ Variations Caused by Structural Changes of the *sn*-2 Carbonyl in DAG-Lactones in the Presence or Absence of Phosphatidyl Serine (PS)

+PS			−PS	
$K_{d(B)}/K_{d(A)}$	$\Delta(\Delta G°)$ (kcal/mol)		$K_{d(B)}/K_{d(A)}$	$\Delta(\Delta G°)$ (kcal/mol)
↓ K_d(CO)/K_d(CO)	0	→	K_d(CO)/K_d(CO)	2.82
↓ K_d(CS)/K_d(CO)	0.74	→	K_d(CS)/K_d(CS)	2.38
↓ K_d(CH_2)/K_d(CO)	3.26	→	K_d(CH_2)/K_d(CH_2)	2.03

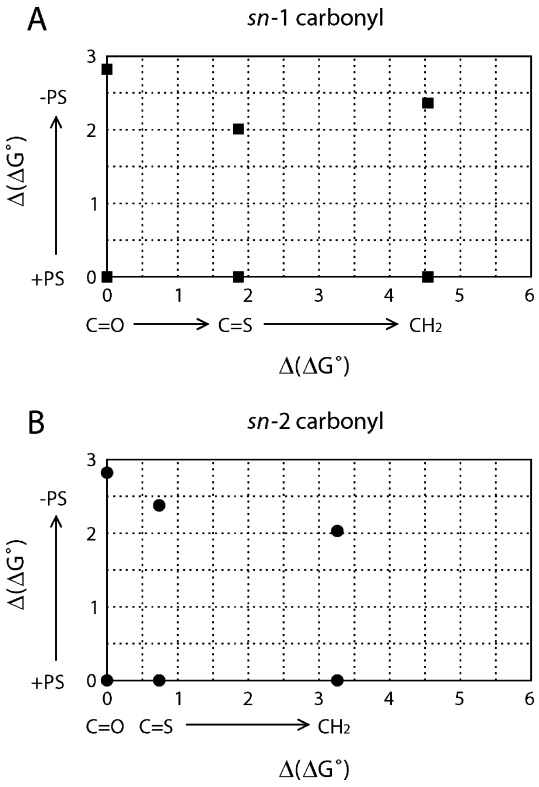


Figure 2. $\Delta(\Delta G°)$ plots for normal and altered *sn*-1 (A) and *sn*-2 (B) carbonyls in the presence or absence of phosphatidyl serine (PS). $\Delta(\Delta G°)$ changes along the *x*-axis correspond to structural changes, while $\Delta(\Delta G°)$ changes along the *y*-axis reflect the effect of PS in the binding process.

Similarly, replacing the carbonyl with a thiocarbonyl also has a more dramatic affect at the *sn*-1 position than at the *sn-2* position (Figure 2A). The effect of removing PS is approximately the same (within 1 kcal/mol) for all the structurally modified DAG-lactones and slightly higher for the parent compound (*E*-**6**).

The parent DAG-lactones, *E*-**6** and *Z*-**7**, were designed to bind exclusively in the *sn*-2 binding mode to ensure the correct orientation and disposition of pharmacophores during binding. In this *sn*-2 binding mode, the *sn*-2 carbonyl is engaged in a hydrogen-bonding interaction with the C1 domain, while the *sn*-1 carbonyl is directed outward toward the solvent environment of the complex. Removing or altering the *sn*-2 carbonyl, therefore, will affect the interactions of the DAG-lactone with the C1 domain, whereas removing or altering the *sn*-1 carbonyl will affect the interactions of the DAG-lactone with surrounding solvent, presumably the interfacial region of the PS bilayer.

These results show that removal of the *sn*-2 carbonyl, as in compound *E*,*Z*-**8** appears to be less costly than removing the *sn*-1 carbonyl, as in compounds *E*-**9** and *Z*-**10**. This suggests that weakening the interaction between the DAG-lactone and its receptor by removing the hydrogen bond formed by the *sn*-2 carbonyl is less important to the overall binding affinity of the complex than altering the interaction of the bound DAG-lactone with the bilayer interface. By use of a similar argument, replacement of the C=O by the less polarized C=S,[25,26] as in compound *E*-**11**, weakens proportionally the strength of the hydrogen bond of the thio-*sn*-2 carbonyl to the C1 domain. However, in the case of the *sn*-1 position, as in compound *E*-**12**, the less hydrated C=S bond interacts less effectively with water or the polar headgroups of the phospholipids. Since very strong hydrogen bonds are formed when one of the partners bears an electrostatic charge, the effect of this change is stronger at the bilayer interface where the *sn*-1 carbonyl resides. The negative effect of removing PS for the parent DAG-lactone (*E*-**6**), as well as the structurally modified compounds, also seems to reflect the importance of the interactions between the DAG-lactone−C1 domain complex and the bilayer interface for productive binding.

The interaction of the *sn*-1 carbonyl on the DAG-lactone with the water and lipid headgroups in the bilayer interface environment may be important for the correct orientation of the molecule at the active site allowing the primary alcohol to engage in hydrogen bonding with Thr242 and Leu251. In the absence of the *sn*-1 carbonyl, the single polar *sn*-2 carbonyl might seek to position itself in the more polar environment of the interface, causing the molecule to flip out of the *sn*-2 binding mode and leading to a very unproductive binding mode with loss of the critical hydrogen bonds to Thr242 and Leu251, which will translate into much higher K_i values.

Additionally or alternatively, the *sn*-1 carbonyl may be important in mediating the penetration of the C1 domain into the membrane as it binds to the DAG-lactones. Before PKC has a chance to access the inner hydrophobic core of the cell membrane, it must first encounter the polar headgroup layer of its constituent phospholipids. Although there have been many experimental and theoretical studies on the energetics of inserting small helical peptides into the bilayer interfacial region, very little work has been done on possible mechanisms for partial β-sheet insertion, as must occur with the C1 domain.[27] Measurements of partitioning of unfolded amino acids between water and POPC bilayers have shown that inserting the backbone amide bond into the lower-dielectric bilayer interface is thermodynamically unfavorable, with a cost of approximately 1.2 kcal/mol for each residue.[28] Yet there are several non-hydrogen-bonded solvent-exposed backbone amide bonds and other polar groups in the C1 domain, even when the ligand is bound (Figure 3A). Several lines of

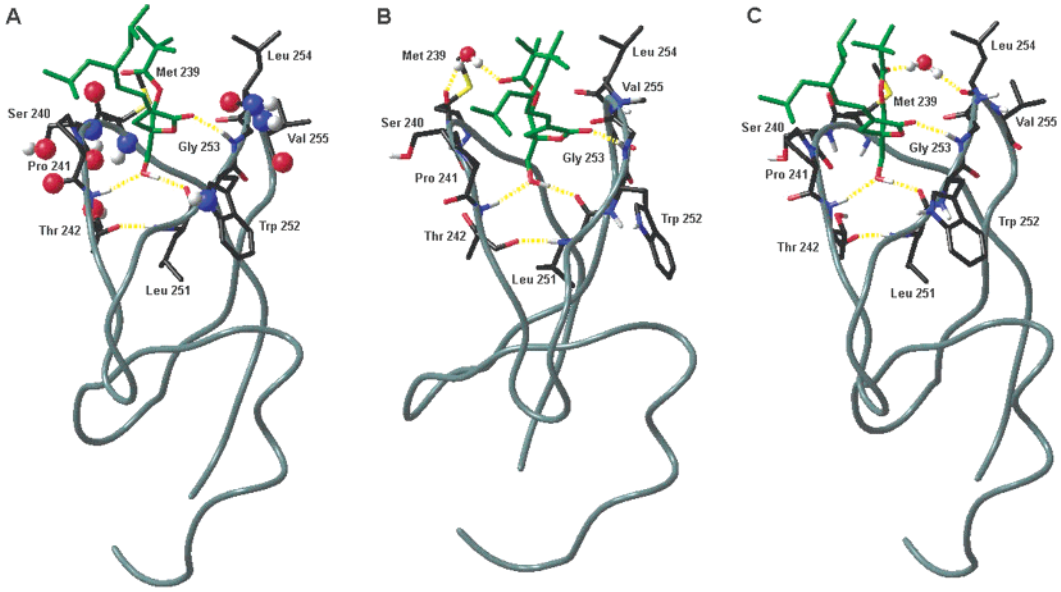


Figure 3. DAG-lactone *E*-**6** (shown in green) docked in the binding site of the C1bδ domain.[13] Hydrogen bonds are drawn with dashed yellow lines. (A) Solvent-exposed non-hydrogen-bonded polar atoms are indicated in larger ball-and-stick representation. (B) Water-bridged hydrogen bonding between the *sn*-1 carbonyl of the DAG-lactone and the backbone carbonyl of residue Met 239. (C) Water-bridged hydrogen bonding between the *sn*-1 carbonyl of the DAG-lactone and the backbone carbonyl of residue Leu 254. Models were built and energy-minimized using the OPLS-2003 force field[30] in MacroModel (Schrödinger, Inc.)

evidence suggest that a certain degree of order is experienced by a few molecules of water that tend to penetrate the membrane's surface. Some experiments suggest that between 5 and 20 water molecules are organized around each molecule of phospholipid.[29] The *sn*-1 carbonyl retains some positional flexibility in the bound complex, and it can orient itself in such a way as to form a water-bridged hydrogen bond to several different backbone carbonyl groups in the C1 domain (Figure 3B,C). It is therefore possible that partial hydration of the *sn*-1 carbonyl, by even one or two water molecules, will provide an energetic advantage for the insertion of the C1 domain by providing prepositioned structural water as hydrogen-binding partners for the backbone amides.

We conclude that the experiments presented here point to the existence of a third unknown binding site, which resides at the lipid interface. Although it is not possible to characterize this binding site in precise structural terms, it appears to be the source of very strong hydrogen-bonding interactions between a hydrated *sn*-1 carbonyl in DAG-lactones (and most likely the C9 OH in the case of phorbol esters) with the charged lipid headgroups and organized water at the lipid interface.

General Experimental Section

All chemical reagents were commercially available. Melting points were determined on a MelTemp II apparatus, Laboratory Devices, and are uncorrected. Column chromatography was performed on silica gel 60, 230−400 mesh (Bodman Ind.), and analytical TLC was performed on Analtech Uniplates silica gel GF. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity Inova instrument at 400 and 100 MHz, respectively. Spectra are referenced to the solvent in which they were run (7.24 ppm for $CDCl_3$). Infrared spectra were recorded on a Jasco model 615 FT-IR instrument. Positive-ion fast atom bombardment mass spectra (FABMS) were obtained on a VG 7070E-HF double-focusing mass spectrometer operating at an accelerating voltage of 6 kV under the control of a MASPEC-II data system for Windows (Mass Spectrometry Services, Ltd.). Either glycerol or 3-nitrobenzyl alcohol was used as the sample matrix, and ionization was effected by a beam of xenon atoms generated in a saddle-field ion gun at 8.0 ± 0.5 kV. Nominal mass spectra were obtained at a resolution of 1200, and matrix-derived ions were background-subtracted during data system processing. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA.

5-Methyl-3-(2-methylpropyl)hexan-1-one (13). According to a general procedure previously reported from this lab,[18] a stirred solution of commercially available 2,6-dimethylheptan-4-one (36 g, 0.25 mol) in THF (200 mL) was cooled to −78 °C and treated dropwise with vinylmagnesium bromide (1 M in THF, 500 mL). The reaction mixture was allowed to reach room temperature and was stirred for 1 h, and the reaction was quenched by the slow addition of a saturated aqueous solution of ammonium chloride (200 mL). The resulting mixture was extracted with ethyl ether (300 mL), and the combined organic extract was washed with water and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel with ethyl ether/hexanes (1:10) as eluant to give intermediate 5-methyl-3-(2-methylpropyl)hex-1-en-3-ol (39 g, 92%) as an oil that was oxidized directly in the next step. A solution of PCC (148 g, 0.69 mol) and 4 Å molecular sieves (148 g) in CH_2Cl_2 (1 L) was treated dropwise with a solution of 5-methyl-3-(2-methylpropyl)hex-1-en-3-ol (39 g 0.23 mol) in CH_2Cl_2 (100 mL). After being stirred for 24 h at room temperature, the reaction mixture was diluted with ethyl ether (500 mL), filtered through a pad of silica gel, and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel with ethyl ether/hexanes (1:10) as eluant to give 5-methyl-3-(2-methylpropyl)hex-2-en-1-one as an oil (38.5 g, 95%), which was then dissolved in CH_2Cl_2 (200 mL) and immediately reduced under a hydrogen-filled balloon in the presence of 10% Pd/C (4 g). After being stirred for 3 h at room temperature, the reaction mixture was filtered through Celite and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel with ethyl ether/hexanes (1:10) as eluant to give 5-methyl-3-(2-methylpropyl)hexan-1-one (**13**) as an oil (23 g, 64%) that was used directly without further purification.

5-Methyl-3-(2-methylpropyl)hexan-1-ol (14). A solution of **13** (12 g, 0.07 mol) in THF (50 mL) was added dropwise over 10 min to a suspension of lithium aluminum hydride (5.3 g, 0.14 mol) in THF (300 mL) that was maintained at 0 °C.

After the addition was complete, the reaction was allowed to reach room temperature. After the mixture was stirred at room temperature for 2 h, the reaction was quenched by the careful addition of ice−water (12 mL), followed by 15% aqueous NaOH (12 mL), and finally a second addition of water (36 mL). The resulting mixture was filtered through Celite and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel with ethyl ether/hexanes (1:5) as eluant to give 5-methyl-3-(2-methylpropyl)hexan-1-ol (**14**) as an oil (10 g, 83%) that was used directly without further purification: ^{1}H NMR ($CDCl_3$) δ 3.55−3.59 (irr t, 2 H, CH_2OH), 1.95 (br s, 1 H, OH), 1.60 (septuplet, 2 H, 2 × CHMe$_2$), 1.45 (m, 3 H, CH_2CH$_2$OH, CH(*i*-Bu)$_2$), 1.05 (irr t, 4 H, 2 × CH_2CHMe$_2$), 0.70−0.85 (singlets, 12 H, 4 × CH$_3$); ^{13}C NMR ($CDCl_3$) δ 61.0, 44.6, 37.6, 30.0, 25.4, 23.2, 22.9.

4-(2-Iodoethyl)-2,6-dimethylheptane (15). 5-Methyl-3-(2-methylpropyl)hexan-1-ol (**14**) (3 g, 0.017 mol) was added to a solution of triphenylphosphine (4.9 g, 0.018 mol) and imidazole (2.5 g, 0.037 mol) in THF (15 mL) and cooled to 0 °C. Dropwise addition of iodine (4.5 g, 0.018 mol) to the resulting suspension at 0 °C was followed by stirring at room temperature for 2 h. The solvent was evaporated in vacuo and the residue was purified by flash column chromatography on silica gel with hexanes as eluant to give 4-(2-iodoethyl)-2,6-dimethylheptane (**15**) as an oil (3.75 g, 78%): ^{1}H NMR ($CDCl_3$) δ 3.21 (t, 2 H, J = 7.6 Hz, CH_2I), 1.75−1.81 (m, 2 H, CH_2CH$_2$I), 1.63 (septuplet, 2 H, 2 × CHMe$_2$), 1.51 (quintuplet, 1 H, CH(*i*-Bu)$_2$), 1.06 (irr t, 2 × CH_2CHMe$_2$), 0.84−0.90 (s, 12 H, 4 × CH$_3$); ^{13}C NMR ($CDCl_3$) δ 43.7, 38.9, 34.3, 25.3, 23.2, 23.0; GC/MS(EI) *m/z* 282 (M^+), 155 (M^+ − I). Anal. ($C_{11}H_{23}I$) C, H, I.

[5-Methyl-3-(2-methylpropyl)hexyl]triphenylphosphonium Iodide (16). A solution of **15** (3.75 g, 0.013 mol) in toluene (10 mL) was treated with triphenylphosphine (5.11 g, 0.019 mol) and heated to reflux for 24 h. After the reaction was completed by TLC analysis, the reaction mixture was concentrated in vacuo. The residue was purified by flash column chromatography on silica gel with EtOAc as eluant to give **16** as a white solid (6.2 g, 84%): mp 55−57 °C; ^{1}H NMR ($CDCl_3$) δ 7.70−7.86 (m, 15 H, Ph), 3.42−3.51 (m, 2 H, CH_2-(PPh_3)$_3$), 1.68 (m, 1 H, CH(*i*-Bu)$_2$), 1.58 (m, 2 H, CH_2CH$_2$-(PPh_3)$_3$), 1.46 (septuplet, 2 H, 2 × CHMe$_2$), 1.14 (m, 4 H, 2 × CH_2CHMe$_2$), 0.87, 0.84, 0.83 and 0.81 (s, 12 H, 4 × CH$_3$); FABMS *m/z* (relative intensity) 417 ($C_{29}H_{38}P^+$, 100.0).

(*E*/*Z*)-4-Methoxy-1-({4-[5-methyl-3-(2-methylpropyl)-hexylidene]-2-[(phenylmethoxy)methyl](2−2,3,5-trihydrofuryl)}methoxy)benzene (18). *n*-Butyllithium (5.2 mL, 2 M in THF, 10.4 mmol) was added to a stirred solution of **16** (4.014 g, 7.4 mmol) in THF (20 mL). After being stirred for 30 min at room temperature, the mixture was cooled to 0 °C and a solution of **17**[20] (1.3 g, 3.7 mmol) in THF (10 mL) was added in one portion. The resulting solution was heated to reflux for 2 h, cooled to room temperature, and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel with hexanes/EtOAc (5:1) as eluant to give **18** as an oil (711 mg, 40%) consisting of an inseparable mixture of geometric isomers. Only the signals for the dominant isomer are reported: ^{1}H NMR ($CDCl_3$) δ 7.24−7.34 (m, 5 H, Ph), 6.79−6.86 (m, 4 H, *Ph*OCH$_3$), 5.30 (m, 1 H, >C=CH), 4.56 (AB q, 2 H, J = 12.3 Hz, OCH_2Ph), 4.43 (br s, 2 H, H-5), 3.95 (AB q, 2 H, J = 9.2 Hz, CH_2OAr), 3.76 (s, 3 H, OCH_3), 3.58 (AB q, 2 H, J = 9.8 Hz, CH_2OBn), 2.48−2.60 (m, 2 H, H-3), 1.96 (irr t, 2 H, >CH=CHCH_2), 1.62 (septuplet, 2 H, 2 × CHMe$_2$), 1.53 (quintuplet, 1 H, CH(*i*-Bu)$_2$), 1.02−1.08 (m, 4 H, 2 × CH_2CHMe$_2$), 0.79−0.87 (m, 12 H, 4 × CH$_3$); ^{13}C NMR ($CDCl_3$) δ 154.0, 153.4, 138.5, 138.3, 134.0, 133.8, 128.9, 128.7, 128.6, 127.7, 127.6, 119.1, 115.8, 114.7, 84.4, 83.7, 73.6, 71.8, 71.7, 71.5, 70.2, 55.9, 44.1, 34.3, 34.2, 33.3, 25.4, 23.2, 22.9; FABMS *m/z* (relative intensity) 481 (MH^+, 19.9), 480 (M^+, 37). Anal. ($C_{31}H_{44}O_4$·0.6H_2O) C, H.

(*E*/*Z*)-{4-[5-Methyl-3-(2-methylpropyl)hexylidene]-2-[(phenylmethoxy)methyl]-2-2,3,5-trihydrofuryl}methan-1-ol (19). A solution of **18** (333 mg, 0.69 mmol) in CH_3CN/H_2O (4:1, 10 mL) was cooled to 0 °C and treated with ammonium cerium(IV) nitrate (1.1 g, 2.1 mmol). After being stirred for 30 min at 0 °C, the reaction mixture was diluted with CH_2Cl_2. The organic layer was washed with water and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel with EtOAc/hexanes (1:2) as eluant to give **19** as an oil (159 mg, 60%) consisting of an inseparable mixture of geometric isomers. Only the signals for the dominant isomer are reported: ^{1}H NMR ($CDCl_3$) δ 7.25−7.36 (m, 5 H, Ph), 5.25−5.29 (m, 1 H, >C=CH), 4.55 (AB q, 2 H, J = 12.3 Hz, OCH_2Ph), 4.37 (br s, 2 H, H-5), 3.65 (dd, 1 H, J = 11.32, 6.64, CHHOH), 3.58 (dd, 1 H, J = 11.32, 6.15, CHHOH), 3.48 (AB q, 2 H, J = 9.4 Hz, CH_2OBn), 2.44 (m, 2 H, H-3), 2.12 (t, 1 H, J = 6.3, OH), 1.94 (irr t, 2 H, >CH=CHCH_2), 1.64 (septuplet, 2 H, 2 × CHMe$_2$), 1.54 (quintuplet, 1 H, CH(*i*-Bu)$_2$), 1.05 (m, 4 H, 2 × CH_2CHMe$_2$), 0.80−0.87 (m, 12 H, 4 × CH$_3$); ^{13}C NMR ($CDCl_3$) δ 138.3, 138.2, 128.5, 127.8, 127.7, 119.2, 113.1, 84.9, 73.8, 72.7, 71.4, 65.5, 44.1, 34.3, 33.7, 33.3, 25.4, 23.2, 22.9; FABMS *m/z* (relative intensity) 375 (MH^+, 12.1). Anal. ($C_{24}H_{38}O_3$·0.1H_2O) C, H.

(*E*/*Z*)-{4-[5-Methyl-3-(2-methylpropyl)hexylidene]-2-[(phenylmethoxy)methyl]-2-2,3,5-trihydrofuryl}methyl 2,2-Dimethylpropanoate (20). A stirred solution of **19** (159 mg, 0.42 mmol) in CH_2Cl_2 (4 mL) at 0 °C was treated with triethylamine (0.18 mL, 1.3 mmol) and pivaloyl chloride (78 μL, 0.63 mmol) for 10 min at the same temperature. Concentration in vacuo followed by flash column chromatography on silica gel with EtOAc/hexanes (1:10) as eluant gave **20** as an oil (152 mg, 82%) consisting of an inseparable mixture of geometric isomers that was used directly in the next step without further purification. Only the signals for the dominant isomer are reported: ^{1}H NMR ($CDCl_3$) δ 7.25−7.35 (m, 5 H, Ph), 5.25−5.30 (m, 1 H, >C=CH), 4.55 (AB q, 2 H, J = 12.3 Hz, OCH_2Ph), 4.40 (m, 2 H, H-5), 4.13 (AB q, 2 H, J = 11.3 Hz, CH_2OCO), 3.45 (AB q, 2 H, J = 9.3 Hz, CH_2OBn), 2.44 (m, 2 H, H-3), 1.94 (irr t, 2 H, >CH=CHCH_2), 1.62 (septuplet, 2 H, 2 × CHMe$_2$), 1.52 (quintuplet, 1 H, CH(*i*-Bu)$_2$), 1.18 (br s, 9 H, CO(CH_3)$_3$), 1.01−1.09 (m, 4 H, 2 × CH_2CHMe$_2$), 0.70−0.99 (m, 12 H, 4 × CH$_3$).

(*E*/*Z*)-{2-(Hydroxymethyl)-4-[5-methyl-3-(2-methylpropyl)hexylidene]-2−2,3,5-trihydrofuryl}methyl 2,2-Dimethylpropanoate (8). A stirred solution of **20** (141 mg, 0.31 mmol) in CH_2Cl_2 (4 mL) was cooled to −78 °C and treated dropwise with BCl_3 (1 M in CH_2Cl_2, 93 μL). After 30 min at −78 °C, the reaction was quenched with a saturated aqueous $NaHCO_3$ solution (10 mL) and the mixture was immediately partitioned between CH_2Cl_2 (10 mL) and the aqueous $NaHCO_3$ solution. The organic layer was washed with water and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel with EtOAc/hexanes (1:2) as eluant to give **8** as an oil (66 mg, 60%) consisting of an inseparable ∼14:1 mixture of geometric isomers. Only the signals for the dominant isomer are reported: ^{1}H NMR ($CDCl_3$) δ 5.75−5.81 (m, 1H, >C=CH), 4.27 (br s, 2 H, H-5), 4.22 (d, 1 H, J = 11.4 Hz, CHHOCO), 3.87 (d, 1 H, J = 11.4 Hz, CHHOCO, 3.38 (broad AB q, 2 H, CH_2OH), 2.63 (br s, 1 H, CH$_2$OH), 2.41 (AB q, 2 H, J = 14.4, H-3), 2.00 (irr t, 2 H, >CH=CHCH_2), 1.62 (septuplet, 2 H, 2 × CHMe$_2$), 1.57 (quintuplet, 1 H, CH(*i*-Bu)$_2$), 1.24 (s, 9 H, CO(CH_3)$_3$), 1.06 (overlapping triplets, 4 H, 2 × CH_2CHMe$_2$), 0.79−0.90 (singlets, 12 H, 4 × CH$_3$); ^{13}C NMR ($CDCl_3$) δ 179.6, 134.9, 132.3, 74.8, 65.8, 65.3, 52.2, 44.2, 39.1, 33.3, 31.5, 27.3, 25.4, 23.2, 23.1, 22.9; FABMS *m/z* (relative intensity) 369 (MH^+, 11.7). Anal. ($C_{22}H_{40}O_4$·0.5H_2O) C, H.

(*E*)-5-[(4-Methoxyphenoxy)methyl]-3-[5-methyl-3-(2-methylpropyl)hexilidene]-5-[(phenylmethoxy)methyl]-4,5-dihydrofuran-2-one (21) and (*Z*)-5-[(4-Methoxyphenoxy)methyl]-3-[5-methyl-3-(2-methylpropyl)hexilidene]-5-[(phenylmethoxy)methyl]-4,5-dihydrofuran-2-one (22). These compounds were synthesized according to previously published methods.[19]

(*Z*)-5-(Hydroxymethyl)-5-[(4-methoxyphenoxy)methyl]-3-[5-methyl-3-(2-methylpropyl)hexylidene]-4,5-dihydrofuran-2-one (24). A stirred solution of **22** (769 mg, 1.5 mmol) in CH_2Cl_2 (8 mL) was cooled to −78 °C and treated dropwise

with BCl_3 (1 M in CH_2Cl_2, 4.5 mL). After 20 min at −78 °C, the reaction was quenched with a saturated aqueous $NaHCO_3$ solution (20 mL) and the mixture was immediately partitioned between CH_2Cl_2 (15 mL) and the aqueous $NaHCO_3$ solution. The organic layer was washed with water and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel with EtOAc/hexanes (1:4) as eluant to give **24** as an oil (530 mg, 86%): ^{1}H NMR ($CDCl_3$) δ 6.84 (s, 4 H, *Ph*OCH$_3$), 6.23−6.28 (tt, 1H, J = 7.6, 2.2 Hz, >C=CH), 4.00 (AB q, 2 H, J = 9.5, Hz, OC*H*$_2$-PhOCH$_3$), 3.80 (br AB q, 2 H, J = 12.1, Hz, C*H*$_2$OH), 3.76 (s, 3 H, OC*H*$_3$), 2.98 (d of irr quartets, 1 H, J = 16.4 Hz, H-4a), 2.89 (d of irr quartets, 1 H, J = 16.4 Hz, H-4b), 2.62−2.74 (m, 2 H, >CH=CHC*H*$_2$), 2.16 (br s, 1 H, CH$_2$O*H*), 1.57−1.71 (overlapping septet and quintet, 3 H, 2 × C*H*Me$_2$, C*H*(*i*-Bu)$_2$), 1.10 (irr t, 4 H, 2 × C*H*$_2$CHMe$_2$), 0.83−0.88 (m, 12 H, 4 × CH$_3$); ^{13}C NMR ($CDCl_3$) δ 169.1, 154.5, 152.6, 144.7, 125.0, 115.8, 114.8, 83.0, 70.3, 65.5, 55.9, 44.1, 33.4, 32.2, 25.3, 23.2, 22.9, 22.8; FABMS *m/z* (relative intensity) 405 (MH$^+$, 57.3), 404 (M$^{\bullet+}$, 100). Anal. ($C_{24}H_{36}O_5$) C, H.

(*Z*)-{2-[(4-Methoxyphenoxy)methyl]-4-[5-methyl-3-(2-methylpropyl)hexylidene]-5-oxo-2−2,3-dihydrofuryl}-methyl Methylsulfonate (26). A solution of **24** (510 mg, 1.3 mmol) in CH_2Cl_2 (5 mL) was cooled to 0 °C, treated with triethylamine (0.54 mL, 3.9 mmol) and methanesulfonyl chloride (0.15 mL, 1.95 mmol), and stirred for 10 min while slowly warming to room temperature. The reaction mixture was concentrated in vacuo and the residue was purified by flash column chromatography on silica gel with EtOAc/hexanes (1:10) as eluant to give **26** as an oil (640 mg, 100%): ^{1}H NMR ($CDCl_3$) δ 6.82 (s, 4 H, *Ph*OCH$_3$), 6.29−6.34 (tt, 1 H, J = 7.6, 2.3 Hz, >C=CH), 4.43 (s, 2 H, C*H*$_2$SO$_2$CH$_3$), 4.01 (AB q, 2 H, J = 9.7, Hz, OC*H*$_2$PhOCH$_3$), 3.76 (s, 3 H, OC*H*$_3$), 3.04 (s, 3 H, SO$_2$C*H*$_3$), 3.02 (d of irr quartets, 1 H, J = 16.6 Hz, H-3a), 2.96 (d of irr quartets, 1 H, J = 16.6 Hz, H-3b), 2.62−2.75 (m, 2 H, >CH=CHC*H*$_2$), 1.58−1.71 (m, 3 H, 2 × C*H*Me$_2$, C*H*(*i*-Bu)$_2$), 1.04−1.16 (m, 4 H, 2 × C*H*$_2$CHMe$_2$), 0.80−0.89 (m, 12 H, 4 × CH$_3$); ^{13}C NMR ($CDCl_3$) δ 168.1, 154.8, 152.1, 145.9, 123.5, 115.8, 114.9, 80.3, 70.3, 70.0, 55.8, 44.1, 37.8, 33.7, 33.4, 32.3, 25.3, 23.2, 22.9, 22.8; FABMS *m/z* (relative intensity) 483 (MH$^+$, 71.5), 482 (M$^{\bullet+}$, 100). Anal. ($C_{25}H_{38}O_7S$) C, H.

(*Z*)-{2-(Hydroxymethyl)-4-[5-methyl-3-(2-methylpropyl)hexylidene-5-oxo-2-2,3-dihydrofuryl}methyl Methylsulfonate (28). A solution of **26** (618 mg, 1.28 mmol) in CH_3CN/H_2O (4:1, 8 mL) was cooled to 0 °C and treated with ammonium cerium(IV) nitrate (2.1 g, 3.84 mmol). After 30 min of being stirred at the same temperature, the reaction mixture was diluted with EtOAc (10 mL). The organic layer was washed with water and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel with EtOAc/hexanes (1:4) as eluant to give **28** as an oil (453 mg, 93%): ^{1}H NMR ($CDCl_3$) δ 6.28−6.33 (tt, 1 H, J = 7.6, 2.3 Hz, >C=CH), 4.36 (AB q, 2 H, J = 11.1 Hz, C*H*$_2$SO$_2$CH$_3$), 3.69−3.76 (m, 2 H, C*H*$_2$OH), 3.07 (s, 3 H, SO$_2$C*H*$_3$), 2.85 (br AB q, 2 H, J = 17.1 Hz, H-3), 2.65−2.71 (m, 2 H, >CH=CHC*H*$_2$), 2.18 (br s, 1 H, CH$_2$O*H*), 1.60−1.71 (m, 3 H, 2 × C*H*Me$_2$, C*H*(*i*-Bu)$_2$), 1.02−1.15 (m, 4 H, 2 × C*H*$_2$CHMe$_2$), 0.85−0.87 (m, 12 H, 4 × CH$_3$); ^{13}C NMR ($CDCl_3$) δ 168.5, 146.0, 123.7, 81.8, 69.8, 64.6, 44.1, 37.7, 33.4, 33.0, 32.3, 25.3, 23.2, 23.1, 22.8; FABMS *m/z* (relative intensity) 377 (MH$^+$, 100.0). Anal. ($C_{18}H_{32}O_6S$) C, H.

(*Z*)-5-[(2,2-Dimethylpropoxy)methyl)-5-(hydroxymethyl)-3-[5-methyl-3-(2-methylpropyl)hexylidene]-4,5-dihydrofuran-2-one (10). A cooled solution of neopentyl alcohol (190 mg, 2.2 mmol) in DMF (5 mL) at 0 °C was treated with triethylamine (0.23 mL, 1.62 mmol). The cooling bath was removed, and after 30 min of stirring at room temperature, a solution of **28** (204 mg, 0.54 mmol) in DMF (5 mL) was added. The resulting solution was stirred for 1 h, and the reaction was quenched by the slow addition of water (5 mL). Following several extractions with EtOAc (40 mL), the combined organic layers were washed with water and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel with ethyl ether/hexanes (1:10) as eluant to give **10** (59 mg, 30%) as an oil: ^{1}H NMR ($CDCl_3$) δ 6.01 (t, 1 H, J = 7.2 Hz, >C=CH), 3.86 (AB q, 2 H, J = 10.7, Hz, C*H*$_2$OCH$_2$C(CH$_3$)$_3$), 3.71 (dd, 1 H, J = 12.3, 4.9 Hz, C*H*HOH), 3.64 (dd, 1 H, J = 12.3, 7.8 Hz CH*H*OH), 2.83 (d, 1 H, J = 4.7 Hz, H-4a), 2.68 (s, 2 H, OC*H*$_2$C(CH$_3$)$_3$), 2.66 (d, 1 H, J = 4.7 Hz, H-4b), 2.46 (irr t, 2 H, $J \approx$ 6.6 Hz, >CH=CHC*H*$_2$), 1.98−2.01 (br dd, $J \approx$ 8.0, 5.2 Hz, 1 H, CH$_2$O*H*), 1.54−1.66 (m, 3 H, 2 × C*H*Me$_2$, C*H*(*i*-Bu)$_2$), 1.06−1.10 (m, 4 H, 2 × C*H*$_2$CHMe$_2$), 0.98 (s, 9 H, OCH$_2$C(C*H*$_3$)$_3$), 0.87 (br s, 6 H, 2 × CH$_3$), 0.85 (br s, 6 H, 2 × CH$_3$); ^{13}C NMR ($CDCl_3$) δ 171.4, 168.5, 145.6, 127.0, 74.4, 63.5, 59.4, 49.7, 44.2, 36.4, 34.5, 33.4, 31.5, 26.8, 25.3, 23.2, 23.2, 22.9; FABMS *m/z* (relative intensity) 369 (MH$^+$, 100.0). Anal. Calcd for $C_{22}H_{40}O_4\cdot 0.8H_2O$: C, 69.00; H, 10.95. Found: C, 68.82; H, 10.28.

(*E*)-5-(Hydroxymethyl)-5-[(4-methoxyphenoxy)methyl]-3-[5-methyl-3-(2-methylpropyl)hexylidene]-4,5-dihydrofuran-2-one (23). Starting from **21** (592 mg, 1.2 mmol) and following the same procedure as for **24**, compound **23** was obtained as an oil (360 mg, 74%): ^{1}H NMR ($CDCl_3$) δ 6.82 (s, 4 H, *Ph*OCH$_3$), 6.78−6.84 (m, 1H, >C=CH), 4.04 (AB q, 2 H, J = 9.6 Hz, OC*H*$_2$Ph), 3.87 (dd, 1 H, J = 12.3, 7.2 Hz, C*H*HOH), 3.78 (dd, 1 H, J = 12.3, 6.4 Hz, CH*H*OH), 3.77 (s, 3 H, OC*H*$_3$), 2.92 (dm, 1 H, J = 16.7 Hz, H-4a), 2.78 (dm, 1 H, J = 16.7 Hz, H-4b), 2.13−2.16 (irr t, 2 H, >CH=CHC*H*$_2$), 1.98 (br t, 1 H, $J \approx$ 7 Hz, CH$_2$O*H*), 1.72 (irr quintet, 1 H, C*H*(*i*-Bu)$_2$), 1.64 (irr septet of doublets, 2 H, 2 × C*H*Me$_2$), 1.05−1.16 (m, 4 H, 2 × C*H*$_2$CHMe$_2$), 0.79−0.89 (m, 12 H, 4 × CH$_3$); ^{13}C NMR ($CDCl_3$) δ 170.1, 154.6, 152.6, 141.1, 127.1, 115.8, 114.8, 83.7, 70.4, 65.6, 55.9, 44.0, 34.9, 33.0, 30.3, 25.4, 23.1, 22.8; FABMS *m/z* (relative intensity) 405 (MH$^+$, 53.8), 404 (M$^+$, 100). Anal. ($C_{24}H_{36}O_5\cdot 0.2H_2O$) C, H.

(*E*)-{2-[(4-Methoxyphenoxy)methyl]-4-[5-methyl-3-(2-methylpropyl)hexylidene]-5-oxo-2-2,3-dihydrofuryl}-methyl Methylsulfonate (25). Starting from **23** (347 mg, 0.86 mmol) and following the same procedure as for **26**, compound **25** was obtained as an oil (415 mg, 100%): ^{1}H NMR ($CDCl_3$) δ 6.79 (s, 4 H, *Ph*OCH$_3$), 6.76−6.84 (m, 1H, >C=CH), 4.41 (s, 2 H, C*H*$_2$SO$_2$CH$_3$), 3.98 (AB q, 2 H, J = 9.7 Hz, OC*H*$_2$Ph), 3.72 (s, 3 H, OC*H*$_3$), 3.01 (s, 3 H, SO$_2$C*H*$_3$), 2.90 (dm, 1H, J = 16 Hz, H-3a), 2.82 (dm, 1 H, J = 16 Hz, H-3b), 2.09−2.14 (m, 2 H, >CH=CHC*H*$_2$), 1.68 (quintuplet, 1 H, C*H*(*i*-Bu)$_2$), 1.59 (m, 2 H, 2 × C*H*Me$_2$), 1.00−1.13 (m, 4 H, 2 × CHC*H*$_2$-CHMe$_2$), 0.76−0.88 (m, 12 H, 4 × CH$_3$); ^{13}C NMR ($CDCl_3$) δ 169.1, 154.8, 152.1, 142.3, 125.6, 115.8, 114.9, 80.9, 70.4, 70.2, 55.8, 44.0, 37.9, 35.1, 33.0, 30.7, 25.4, 23.1, 22.8, 22.7; FABMS *m/z* (relative intensity) 483 (MH$^+$, 62.0), 482 (M$^{\bullet+}$, 100). Anal. ($C_{25}H_{38}O_7S$) C, H.

(*E*)-{2-(Hydroxymethyl)-4-[5-methyl-3-(2-methylpropyl)hexylidene-5-oxo-2-2,3-dihydrofuryl}methyl Methylsulfonate (27). Starting from **25** (415 mg, 0.86 mmol) and following the same procedure as for **28**, compound **27** was obtained as an oil (271 mg, 84%): ^{1}H NMR ($CDCl_3$) δ 6.72−6.77 (m, 1 H, >C=CH), 4.30 (AB q, 2 H, J = 11.1 Hz, C*H*$_2$SO$_2$CH$_3$), 3.68 (br s, 2 H, C*H*$_2$OH), 3.03 (s, 3 H, SO$_2$C*H*$_3$), 2.94 (br s, 1 H, CH$_2$O*H*), 2.71 (m, 2 H, H-3), 2.07 (m, 2 H, >CH=CHC*H*$_2$), 1.66 (irr quintet, 1 H, C*H*(*i*-Bu)$_2$), 1.57 (irr septet, 2 H, 2 × C*H*Me$_2$), 0.97−1.10 (m, 4 H, 2 × C*H*$_2$CHMe$_2$), 0.75−0.85 (m, 12 H, 4 × CH$_3$); ^{13}C NMR ($CDCl_3$) δ 169.7, 142.3, 126.0, 82.7, 70.0, 64.7, 44.0, 37.7, 35.1, 32.9, 29.9, 25.4, 23.1, 22.8, 22.7; FABMS *m/z* (relative intensity) 377 (MH$^+$, 100.0). Anal. ($C_{18}H_{32}O_6S$) C, H.

(*E*)-5-[(2,2-Dimethylpropoxy)methyl)-5-(hydroxymethyl)-3-[5-methyl-3-(2-methylpropyl)hexylidene]-4,5-dihydrofuran-2-one (9). Starting from **27** (147 mg, 0.39 mmol) and following the same procedure as for **10**, compound **9** (100 mg, 70%) was obtained as an oil: ^{1}H NMR (DMSO) δ 6.78 (t, 1 H, J = 7.2 Hz, >C=CH), 4.81 (t, 1 H, J = 6.0 Hz, CH$_2$O*H*), 3.71 (AB q, 2 H, J = 10.5, Hz, C*H*$_2$OCH$_2$C(CH$_3$)$_3$), 3.43 (dd, 1 H, J = 11.9, 5.8, C*H*HOH), 3.32 (dd, 1 H, J = 11.9, 6.0 Hz, CH*H*OH), 2.67 (AB q, 2 H, J = 14.2, Hz, OC*H*$_2$C(CH$_3$)$_3$), 2.51 (d, 1 H, J = 5.1 Hz, H-4a), 2.28 (d, 1 H, J = 5.1 Hz, H-4b), 2.09 (irr t, 2 H, $J \approx$ 6.7 Hz, >CH=CHC*H*$_2$), 1.48−1.61 (m, 3 H, 2 × C*H*Me$_2$, C*H*(*i*-Bu)$_2$), 0.93−1.05 (irr t, $J \approx$ 7.0 Hz, 4 H, 2 × C*H*$_2$CHMe$_2$), 0.86 (s, 9 H, OCH$_2$C(C*H*$_3$)$_3$), 0.77−0.79

(singlets, 12 H, 4 × CH_3); ^{13}C NMR ($CDCl_3$) δ 168.4, 146.5, 127.2, 74.3, 64.0, 59.5, 50.4, 44.5, 44.4, 34.0, 33.2, 31.7, 28.6, 26.6, 25.4, 23.1, 22.8; FABMS *m/z* (relative intensity) 369 (MH^+, 42.9). Anal. ($C_{22}H_{40}O_4$) C, H.

5-(Hydroxymethyl)-5-[(phenylmethoxy)methyl]-3,4,5-trihydrofuran-2-one (30). A stirred solution of **29**[19] (3.3 g, 9.6 mmol) in CH_3CN/H_2O (4:1, 20 mL) was cooled to 0 °C and treated with ammonium cerium(IV) nitrate (15.0 g, 28.8 mmol). After being stirred for an additional 30 min at the same temperature, the reaction mixture was diluted with CH_2Cl_2 and the organic layer was washed with water and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel with EtOAc/hexanes (1:2) as eluant to give **30** as an oil (1.76 g, 78%): ^1H NMR ($CDCl_3$) δ 7.25−7.36 (m, 5 H, Ph), 4.54 (s, 2 H, OCH_2Ph), 3.75 (dd, 1 H, J = 12.1, 6.4 Hz, C*H*HOH), 3.62 (dd, 1 H, J = 12.1, 6.4 Hz, CH*H*OH), 3.55 (AB q, 2 H, J = 10.3 Hz, CH_2OBn), 2.96 (t, 1 H, J = 6.4 Hz, CH_2O*H*), 2.53−2.69 (m, 2 H, H-3), 2.09−2.20 (m, 2 H, H-4); ^{13}C NMR ($CDCl_3$) δ 177.8,137.7, 128.7, 128.0, 127.8, 88.0, 73.8, 72.6, 65.5, 29.4, 25.8. FABMS *m/z* (relative intensity) 237 (MH^+, 20.9). Anal. ($C_{13}H_{16}O_4$) C, H.

5,5-Bis[(phenylmethoxy)methyl]-3,4,5-trihydrofuran-2-one (31). A solution of **30** (1.76 g, 7.5 mmol) in DMF (10 mL) at 0 °C was treated with NaH (60% dispersion in mineral oil, 600 mg, 15.0 mmol). After removal of the cooling bath, stirring continued for 10 min at room temperature and then benzyl bromide (1.1 mL, 9.0 mmol) was added. The resulting solution was stirred for 1 h, and then the reaction was quenched by slow addition of water (70 mL). The sample was extracted with EtOAc (50 mL) several times. The combined organic layers were washed with water and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel with ethyl ether/hexanes (1:20) as eluant to give **31** (1.2 g, 50%) as an oil: ^1H NMR ($CDCl_3$) δ 7.26−7.38 (m, 5 H, Ph), 4.55 (s, 4 H, OCH_2Ph), 3.58 (AB q, 4 H, J = 10.3 Hz, CH_2OBn), 2.60 (irr t, 2 H, $J \approx$ 8.6 Hz, H-3), 2.15 (irr t, 2 H, $J \approx$ 8.6 Hz, H-4). This compound was used in the next step without further purification.

(*E*)-5,5-Bis[phenylmethoxy)methyl]-3-[5-methyl-3-(2-methylpropyl)hexylidene]-4,5-dihydrofuran-2-one (32) and (*Z*)-5,5-Bis[phenylmethoxy)methyl]-3-[5-methyl-3-(2-methylpropyl)hexylidene]-4,5-dihydrofuran-2-one (33). A stirred solution of **31** (1.2 g, 3.8 mmol) in THF (10 mL) was cooled to −78 °C and treated dropwise with lithium bis-(trimethylsilyl)amide (1 M in THF, 7.6 mL). After being stirred for an additional 30 min at −78 °C, the mixture was treated with a solution of **13** (647 mg, 3.8 mmol) in THF (10 mL) and stirred for 1 h at the same temperature. The reaction was quenched by the slow addition of saturated aqueous NH_4Cl (20 mL), and the sample was extracted with ether (20 mL) several times. The combined organic layers were washed with water and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel with EtOAc/hexanes (1:10) as eluant to give the intermediate β-hydroxylactone as an oil (1.47 g, 2.9 mmol), which was then immediately dissolved in CH_2Cl_2 (10 mL), cooled to 0 °C, and treated with triethylamine (1.2 mL, 8.7 mmol) and methanesulfonyl chloride (0.34 mL, 4.4 mmol). After the mixture was warmed to room temperature and further stirred at room temperature for 10 min, 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU, 1.3 mL, 8.7 mmol) was added and the resulting solution was stirred for a total of 15 min. The reaction mixture was concentrated in vacuo and the residue was purified by flash column chromatography on silica gel with EtOAc/hexanes (1:10) as eluant to give **32** (425 mg, 31%) and **33** (599 mg, 45%) as oils.

32: ^1H NMR ($CDCl_3$) δ 7.25−7.36 (m, 5 H, Ph), 6.72−6.77 (br tt, J = 7.6 Hz, 1 H, >C=CH), 4.56 (AB s, 4 H, 2 × OCH_2Ph), 3.58 (AB q, 4 H, J = 10.1 Hz, 2 × CH_2OBn), 2.74 (br s, 2 H, H-4), 2.10 (t, 2 H, >CH=CHCH_2), 1.56−1.72 (m, 3 H, 2 × C*H*Me_2, C*H*(i-Bu)$_2$), 1.08 (t, 4 H, J = 7.0 Hz, 2 × CH_2CHMe_2), 0.80−0.89 (m, 12 H, 4 × CH_3); FABMS *m/z* (relative intensity) 479 (MH^+, 1.7). Anal. ($C_{31}H_{42}O_4 \cdot 0.4H_2O$) C, H.

33: ^1H NMR ($CDCl_3$) δ 7.25−7.35 (m, 5 H, Ph), 6.12−6.18 (tt, 1 H, J = 7.5, 2.3 Hz, >C=CH), 4.56 (AB s, 4 H, 2 × OCH_2Ph), 3.57 (AB q, 4 H, J = 10.1 Hz, 2 × CH_2OBn), 2.82 (AB q, J = 2.2 Hz, 2 H, H-4), 2.64−2.70 (m, 2 H, >CH=CHCH_2), 1.56−1.72 (m, 3 H, 2 × C*H*Me_2, C*H*(i-Bu)$_2$), 1.08 (irr t, $J \approx$ 7.1 Hz, 4 H, 2 × CH_2CHMe_2), 0.78−0.88 (m, 12 H, 4 × CH_3); FABMS *m/z* (relative intensity) 479 (MH^+, 1.1). Anal. ($C_{31}H_{42}O_4 \cdot 0.2H_2O$) C, H.

(*E*)-5,5-Bis[(phenylmethoxy)methyl]-3-[5-methyl-3-(2-methylpropyl)hexylidene]-4,5-dihydrofuran-2-thione (34). Lawesson's reagent (2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulfide, 1.0 g, 2.6 mmol) and **32** (or **33**) (599 mg, 1.3 mmol) were dissolved in toluene (6 mL) and stirred for 18 h at 110 °C. After cooling to room temperature, the solution was concentrated under reduced pressure until an oily material appeared, and then hexane was added to induce precipitation of excess Lawesson's reagent. The precipitate was removed by filtration, and the filtrate was evaporated to dryness. Hexane was added again to the residue, and the above filtration−evaporation process was repeated twice. The resulting residue was then subjected to column chromatography to give **34** (470 mg, 73%) as an oil: ^1H NMR ($CDCl_3$) δ 7.25−7.36 (m, 5 H, Ph), 7.05−7.10 (tt, 1H, J = 7.8, 2.7 Hz, >C=CH), 4.57 (s, 4 H, 2 × OCH_2Ph), 3.64 (AB q, 4 H, J = 10.3 Hz, 2 × CH_2OBn), 2.86 (br s, 2 H, H-4), 2.09−2.12 (irr dd, 2 H, >CH=CHCH_2), 1.75 (irr quintet, 1 H, C*H*(i-Bu)$_2$), 1.64 (irr septet, 2 H, 2 × C*H*Me_2), 1.09−1.17 (m, 4 H, 2 × CH_2CHMe_2), 0.80−0.95 (m, 12 H, 4 × CH_3); ^{13}C NMR ($CDCl_3$) δ 211.4, 142.9, 139.1, 137.6, 128.4, 127.7, 127.6, 92.4, 73.7, 71.5, 44.1, 35.8, 33.1, 31.4, 25.3, 22.9, 22.6; FABMS *m/z* (relative intensity) 495 (MH^+, 2.7). Anal. ($C_{31}H_{42}O_3S \cdot 1.1H_2O$) C, H.

(*E*)-{2-(Hydroxymethyl)-4-[5-methyl-3-(2-methylpropyl)hexylidene]-5-thioxo-2-2,3-dihydrofuryl}methyl 2,2-Dimethylpropanoate (11). A stirred solution of **34** (470 mg, 0.95 mmol) in CH_2Cl_2 (10 mL) was cooled to −78 °C and treated dropwise with BCl_3 (1 M solution in CH_2Cl_2, 5.7 mL). After being stirred for an additional 30 min at the same temperature, the reaction was quenched by the addition of an aqueous saturated $NaHCO_3$ solution (10 mL). The mixture was warmed to room temperature and immediately partitioned between ether and the aqueous $NaHCO_3$ solution. The organic layer was washed with water and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel with EtOAc/hexanes (1:2) as eluant to give a diol intermediate as a clear oil (242 mg, 81%) that was used immediately in the following step.

A stirred solution of the diol (71 mg, 0.54 mmol) in CH_2Cl_2 (2 mL) was cooled to 0 °C and treated with triethylamine (48 μL, 0.35 mmol) and pivaloyl chloride (31 μL, 0.25 mmol) for 10 min. The reaction mixture was then concentrated in vacuo and the residue was purified by flash column chromatography on silica gel with EtOAc/hexanes (1:10) as eluant to give **11** as a yellowish oil (59 mg, 65%): ^1H NMR ($CDCl_3$) δ 7.06−7.11 (tt, 1H, J = 7.7, 2.8 Hz, >C=CH), 4.37 (d, 1 H, J = 12.1, Hz, C*H*HOCO), 4.20 (d, 1 H, J = 12.1, Hz, CH*H*OCO), 3.76 (AB q, 2 H, J = 12.3, Hz, CH_2OH), 2.91 (dm, 1 H, $J \approx$ 16.5 Hz, H-3a), 2.76 (dm, 1 H, $J \approx$ 16.5 Hz, H-3b), 2.30−2.40 (br s, 1 H, CH_2O*H*), 2.09−2.13 (m, 2 H, >CH=CHCH_2), 1.75 (irr quintet, 1 H, C*H*(i-Bu)$_2$), 1.63 (irr septet, 2 H, 2 × C*H*Me_2), 1.18 (s, 9 H, COCH(CH_3)$_3$), 1.07−1.18 (m, 4 H, 2 × CH_2CHMe_2), 0.86−0.90 (m, 12 H, 4 × CH_3); ^{13}C NMR ($CDCl_3$) δ 210, 178.1, 143.9, 138.4, 91.8, 65.1, 64.4, 44.1, 35.9, 33.0, 30.9, 27.0, 25.3, 25.2, 22.9, 22.6; FABMS *m/z* (relative intensity) 399 (MH^+, 20.1). Anal. ($C_{22}H_{38}O_4S \cdot 0.33H_2O$) C, H.

(*E*)-5-[(2,2-Dimethyl-1-thioxopropoxy)methyl]-5-(hydroxymethyl)-3-[5-methyl-3-(2-methylpropyl)hexylidene]-4,5-dihydrofuran-2-one (12). BCl_3 (1 M in CH_2Cl_2, 5.3 mL) was added slowly to a −78 °C solution of **32** (425 mg, 0.89 mmol) in CH_2Cl_2 (10 mL) and stirred for 1 h. Aqueous saturated $NaHCO_3$ (10 mL) was added slowly, and the mixture was then diluted with CH_2Cl_2 (4 mL). The layers were separated, and the aqueous layer was further extracted with CH_2Cl_2. The combined organic extract was dried ($MgSO_4$) and concentrated in vacuo, and the residue was purified by silica

gel column chromatography to give diol **35** (200 mg, 75%) as a clear oil that was used directly for the following step. FABMS *m/z* (relative intensity) 299 (MH^+, 100).

A solution of **35** (59 mg, 0.2 mmol) in THF (10 mL) at room temperature was treated with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 56 μL, 0.40 mmol) and 2,2-dimethyl-1-(6-nitrobenzotriazolyl)propane-1-thione (**36**)[21] (53 mg, 0.25 mmol) and stirred for 10 min. The reaction mixture was concentrated in vacuo and the residue was purified by flash column chromatography on silica gel with EtOAc/hexanes (1:40) as eluant to give **12** as a yellowish oil (50 mg, 60%): ^{1}H NMR ($CDCl_3$) δ 6.80−6.85 (tt, 1 H, J = 7.6, 2.8 Hz, 1 H, >C=CH), 4.55 (AB q, 2 H, J = 11.9 Hz, CH_2OCS), 3.84 (dd, 1 H, J = 12.1, 6.4 Hz, C*H*HOH), 3.71 (dd, 1 H, J = 12.1, 6.4 Hz, CH*H*OH), 2.87 (dm, 1 H, $J \approx$ 12.5 Hz, H-4a), 2.73 (dm, 1 H, $J \approx$ 12.5 Hz, H-4b), 2.19 (t, 1 H, J = 6.4 Hz, CH_2OH), 2.13 (m, 2 H, >CH=CHCH_2), 1.72 (irr quintet, 1 H, C*H*(*i*-Bu)$_2$), 1.64 (irr septet, 2 H, 2 × C*H*Me$_2$), 1.27 (s, 9 H, CS(CH_3)$_3$), 1.05−1.15 (m, 4 H, 2 × CH_2CHMe$_2$), 0.80−0.90 (m, 12 H, 4 × CH_3); FABMS *m/z* (relative intensity) 399 (MH^+, 28.2), 101 ($(CH_3)_3C{\equiv}S^+$, 66). Anal. ($C_{22}H_{38}O_4S$) C, H.

[^{3}H]PDBu Binding Assay. [^{3}H]PDBu was obtained from Perkin-Elmer (no longer available as a catalog item). PDBu was purchased from LC Laboratories (Woburn, MA). The recombinant full-length PKCα was purchased from Invitrogen (Carlsbad, CA). The recombinant plasmid of GST-δC1b was constructed as described previously.[31] The C1bδ protein was expressed and purified from BL-21 (DE3) *E. coli* (Stratagene, La Jolla, LA) using a B-PER GST spin purification kit (Pierce Biotechnology, Rockford, IL), according to the manufacturer's instruction. The purity of the protein was verified by SDS−PAGE and staining with Coomassie Blue.

Binding of [^{3}H]PDBu to PKCα and the isolated δC1b domain was measured using the poly(ethylene glycol) precipitation assay.[23] Briefly, an assay mixture (250 μL) containing 50 mM Tris-HCl (pH 7.4), 100 μg/mL phosphatidylserine (PS), 4 mg/mL bovine IgG, [^{3}H]PDBu, 0.1 mM $CaCl_2$ (for PKCα, or 1 mM EGTA for C1bδ), various concentrations of competing ligand, and the receptor was incubated for 5 min at 37 °C (for PKCα) or 10 min at 18 °C (for C1bδ). The samples were then chilled on ice for 7 min, and 200 μL of 35% poly(ethylene glycol) in 50 mM Tris-HCl (pH 7.4) was added. The tubes were incubated on ice for an additional 10 min and then centrifuged at 4 °C. A 100 μL aliquot of the supernatant was removed for the determination of the free [^{3}H]PDBu concentration, and the pellet was carefully dried. The tip of the tube was cut off, and the pellet was counted in a scintillation counter to determine the total bound [^{3}H]PDBu. Specific binding was calculated as the difference between the total and the nonspecific binding, which was determined in the presence of 64 μM nonradioactive PDBu. For measuring the competitive binding of the compound with [^{3}H]PDBu in the absence of PS, a similar protocol was used except that the PS was omitted.

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Supporting Information Available: Combustion analysis for compounds **8**−**12**, **15**, **18**, **19**, **23**−**28**, **30**, and **32**−**34**. This material is available free of charge via the Internet at http://pubs.acs.org.

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