# Conformationally Constrained Analogues of Diacylglycerol (DAG). 23†. Hydrophobic Ligand−Protein Interactions versus Ligand−Lipid Interactions of DAG-Lactones with Protein Kinase C (PK-C)

Hirokazu Tamamura,[‡,§] Dina M. Sigano,[‡] Nancy E. Lewin,[||] Megan L. Peach,[‡] Marc C. Nicklaus,[‡] Peter M. Blumberg,[||] and Victor E. Marquez*[,‡]

*Laboratory of Medicinal Chemistry, Center for Cancer Research, National Cancer Institute−Frederick, National Institutes of Health, Building 376, Room 104, Frederick, Maryland 21702, and Laboratory of Cellular Carcinogenesis & Tumor Promotion, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Building 37, Room 3A01, Bethesda, Maryland 20892*

*Received April 12, 2004*

The constrained glycerol backbone of DAG-lactones, when combined with highly branched alkyl chains, has engendered a series of DAG-lactone ligands capable of binding protein kinase C (PK-C) with affinities that approximate those of phorbol esters. These branched chains not only appear to be involved in making important hydrophobic contacts with the protein (specific interactions) but also provide adequate lipophilicity to facilitate partitioning into the lipid-rich membrane environment (nonspecific interactions). With the idea of minimizing the nonspecific interactions without reducing lipophilicity, the present work explores the strategy of relocating lipophilicity from the side chain to the lactone "core". Such a transfer of lipophilicity, exemplified by compounds **1** and **3**, was conceived to allow the new hydrophobic groups on the lactone to engage in specific hydrophobic contacts inside the binding pocket without any expectation of interfering with the hydrogen-bonding network of the DAG-lactone pharmacophore. Surprisingly, both (*E*)-**3** and (*Z*)-**3** showed a significant decrease in binding affinity. From the molecular docking studies performed with the new ligands, we conclude that the binding pocket of the C1 domain of PK-C is sterically restricted and prevents the methyl groups at the C-3 position of the lactone from engaging in productive hydrophobic contacts with the receptor.

## Introduction and Background

The central role of protein kinase C (PK-C) in cell-signal transduction has been well established over the past two decades since its discovery.[1,2] The PK-C family is comprised of 10 isozymes grouped into 3 classes: conventional (α, $\beta$1 and $\beta$2, $\gamma$), novel ($\delta$, $\epsilon$, $\eta$, and $\theta$), and atypical ($\zeta$ and $\iota/\lambda$). In addition, PK-C $\mu$ and $\nu$ are considered by some to constitute a fourth class and by others to compose a distinct family called protein kinase D.[2] All isozymes contain a C-terminal kinase domain with serine/threonine specific kinase activity and an N-terminal regulatory domain. The regulatory domain contains two key functionalities: an autoinhibitory sequence and one or two membrane-targeting domains (C1 and C2).

Both classical and novel PK-C isozymes are thought to become activated as a result of association of the cytosolic enzyme with membranes containing acid phospholipids.[3,4] This association is strongly facilitated by the second messenger, *sn*-1,2-diacylglycerol (DAG), which is generated as a result of a stimulus-initiated activation of phospholipase C.[5] The accepted mechanism is that these isozymes are cytosolic in the inactive state and translocate to the inner leaflet of the cellular membrane as part of the activation process.[6,7] The binding of PK-C to the plasma membrane is transient and is regulated by the association of its C1 domain with DAG in the membrane.[8]

These C1 domains in the classical and novel PK-C isozymes contain two small (∼50 residues) zinc-finger-like structures (C1a and C1b), consisting of two $\beta$ sheets and a small α helix. The crystal structure of a single C1b domain from PK-C$\delta$ in complex with the PK-C activator, phorbol-13-*O*-acetate,[9] revealed that the ligand is bound inside the pocket between the two hydrophobic $\beta$ sheets, thus capping the C1b domain and forming a continuous hydrophobic surface that promotes the insertion of the binary complex into the membrane. This X-ray structure also confirmed the importance of hydrogen bonding of the main phorbol ester pharmacophores (C-3, C-4, and C-20) with amino acids inside the binding pocket and furthermore revealed that the lipophilic acyl chains at C-12 and C-13 are important for activity because they are projected into the proper position to interact with membrane lipids in the ternary complex.[10−13]

Docking of DAG into the same empty C1b domain revealed a similar pattern of hydrogen bonding involving pharmacophores at C-1, C-2, and C-3. Modeling

* Author to whom correspondence should be addressed. Tel: 301-846-5954. Fax: 301-846-6033. E-mail: marquezv@mail.nih.gov.

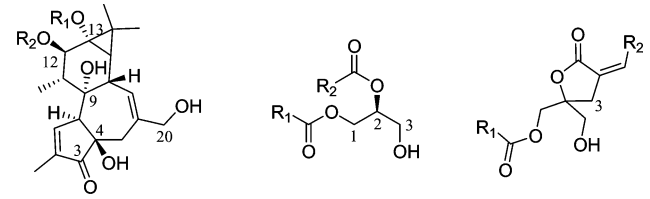
† Because of a clerical error resulting in two publications designated as number 19, there is no paper no. 22. See Nacro, K. et al. Conformationally constrained analogues of diacylglycerol (DAG). Part 19: Asymmetric syntheses of (3R)- and (3S)-3-hydroxy-4,4- disubstituted heptono-1,4-lactones as protein kinase C (PK−C) ligands with increased hydrophilicity. *Tetrahedron* **2002**, *58*, 5335−5345 and Choi, Y. et al. Conformationally constrained analogues of diacylglycerol. 19. Synthesis and protein kinase C binding affinity of diacylglycerol lactones bearing an N-hydroxylamide side chain. *J. Med. Chem.* **2003**, *46*, 2790−2793. Paper nos. 20 and #21 have already been published.

‡ Laboratory of Medicinal Chemistry.

§ Present address: Graduate School of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto 606-8501, Japan.

|| Laboratory of Cellular Carcinogenesis & Tumor Promotion.

10.1021/jm049723+ 
Published on Web 08/27/2004



**phorbol: $R_1 = R_2 = H$** ***sn*-1,2-diacylglycerol (DAG)** **DAG-Lactone**

**PMA:** $R_1 = C(O)CH_3$, $R_2 = C(O)C_{13}H_{27}$

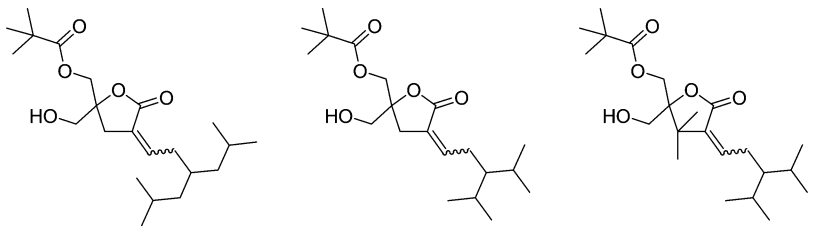
**Figure 1.** Structures of phorbol esters, DAG, and DAG-lactone.

alone, however, does not explain the substantial difference in binding affinity (≥3 orders of magnitude) that exists between phorbol esters and DAG (Figure 1).[14,15]

Over the past few years, we have attempted to bridge this affinity gap between phorbol esters and DAGs by two independent approaches. One approach includes reducing the entropic penalty associated with DAG binding by constraining the glycerol backbone into DAG-lactones (Figure 1).[16,17] A second approach involves increasing the bulk of the acyl and α-alkylidene groups in these DAG-lactones by incorporating highly branched alkyl chains that are capable of making important hydrophobic contacts with a group of conserved hydrophobic amino acids in the space between the two $\beta$ sheets of the C1b domain.[16−19] An additional role for these alkyl chains is to provide adequate lipophilicity, expressed as a log *P* value, to facilitate partitioning into the lipid-rich membrane environment of the cell. The log *P* (octanol/water partition coefficient) is a measure of the hydrophobic/hydrophilic balance of a molecule, and in the present work, it is calculated by the fragment-based program KOWWIN 1.67.[20,21] Because of the dual role of the alkyl chains, a higher binding affinity due to increased lipophilicity is probably the result of these two important factors: adequate membrane partitioning (nonspecific interactions) and hydrophobic contacts with the protein (specific interactions). To understand this complex relationship between affinity (expressed as $K_i$) and hydrophobicity (log *P*), we have examined plots of $1/K_i$ versus log *P* and determined that the optimal log *P* for DAG-lactones lies somewhere between 5 and 6.[18] With the idea of reducing the nonspecific interactions of the side chains without dropping below the minimum log *P* threshold required for adequate membrane distribution, we decided to explore a strategy of transferring lipophilic methyl groups from the side chain to the lactone "core". Thus, compound **3** was designed on the basis of the properties of DAG-lactones, **1** and **2**, which are known as effective PK-C ligands with affinities in the low nanomolar range (Figure 2). The removal of two carbons from the side chain of **1** (log $P = 5.03$) to give **2** (log $P = 4.04$) reduced the log *P* by almost 1 unit and caused a ca. 2.5-fold reduction in PK-Cα binding affinity. If instead of removing two carbon units from the side chain of **1** we transfer them to the C-3 position on the lactone ring to give **3**, then one would maintain a nearly identical log *P* (log $P = 4.91$). In addition, if the receptor was flexible enough to accommodate the ligand in the usual manner, then perhaps this would allow the new hydrophobic groups on the lactone to engage in specific hydrophobic contacts inside the binding pocket, without any expectation of interfering with the hydrogen-bonding network. The aim of the present work was to synthesize target compound **3** and test the basis of this hypothesis.

## Results and Discussion

**Chemistry.** The essential methyl groups on the lactone ring in **3** were introduced early in the synthesis via a radical exo-trig cyclization from intermediate **4** (Scheme 1). This approach was based on the work of Ueno and co-workers, who developed a particularly useful synthesis of $\gamma$-butyrolactones via the stereoselective radical cyclization of bromoacetals in the presence of AIBN and tin hydride.[22] Ueno's methodology has been considered to be particularly advantageous in cases such as ours, where a sterically hindered carbon is involved because it is known that this type of carbon−carbon bond-forming reaction generally gives poor results. The exclusive exo-trig cyclization anticipated from the Baldwin rules for ring closure ensured an efficient route to our functionalized $\gamma$-butyrolactones. Racemic intermediate **5**, obtained from the Grignard reaction of 1-(benzyloxy)-3-(4-methoxyphenoxy)acetone[23] and isopropenyl bromide, provided the requisite bromoacetal (**6**) via alkoxybromination with ethyl vinyl ether in the presence of NBS. Immediate radical cyclization led to the dimethylated ethyl glycoside (**7**) in excellent yield. Hydrolysis to the lactol (**8**) followed by oxidation gave the essential dimethyl lactone (**9**). Aldol condensation of **9** with 4-methyl-3-(methylethyl)pentan-1-one using LDA gave the expected aldol product, but elimination to the olefin using our standard protocol proved difficult. Indeed, mesylation followed by attempted DBU elimination gave a mixture of unreacted alcohol, the corresponding mesylate ester, and the lactone product of the reverse aldol reaction with no elimination products observed. However, when this reaction mixture was further treated with NaH in DMF, the desired α-alky-



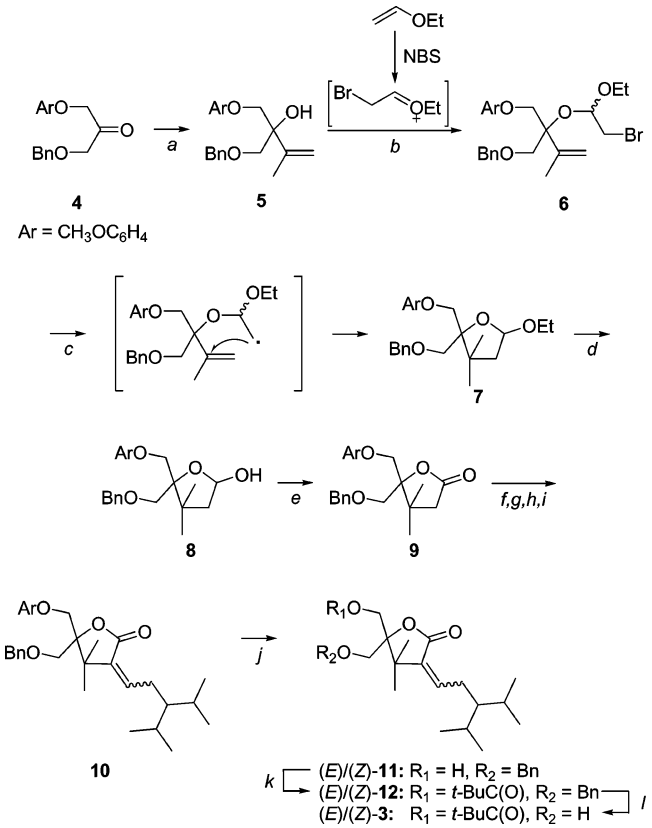
**1**
log P = 5.03
E: Ki = 3.25 +/- 0.15
Z: Ki = 2.9 +/- 0.35

**2**
log P = 4.04
E: Ki = 11.25 +/- 0.7
Z: Ki = 8.32 +/- 0.7

**3**
log P = 4.91

**Figure 2.** Structures of DAG-lactones **1**−**3** showing their corresponding log *P* values and PK-Cα binding affinities ($K_i$) for compounds **1** and **2**.

**Scheme 1**[a]




[a] (a) isopropenylmagnesium bromide, THF, −78 → −25 °C (50%); (b) ethyl vinyl ether, 4-Å molecular sieves, NBS, $CH_2Cl_2$, 0 °C (22%); (c) *n*-$Bu_3SnH$, AIBN, $C_6H_6$, Δ (91%); (d) HCl, THF, $H_2O$ 0 °C (77%); (e) TPAP, NMO, 4Å ms, $CH_2Cl_2/CH_3CN$, 0 °C → rt (66%); (f) LDA, *i*-$Pr_2CHCH_2CHO$, THF, −78 °C; (g) MsCl, $Et_3N$, $CH_2Cl_2$, 0 °C → rt; (h) DBU, 0 °C → rt; (i) NaH, DMF (26% (*Z*)-**10** and 13% (*E*)-**10** for four steps); (j) CAN, $CH_3CN/H_2O$, 0 °C (72% (*Z*)-**11** and 57% (*E*)-**11**); (k) $(CH_3)_3C(O)Cl$, DMAP, pyridine (88% (*Z*)-**12** and 92% (*E*)-**12**); (l) $BCl_3$, $CH_2Cl_2$, −78 °C (92% (*Z*)-**3** and 93% (*E*)-**3**).

lidene DAG-lactone **10** was obtained as a mixture of *E* and *Z* isomers, which were easily separated by silica gel column chromatography. Consistent with previously synthesized DAG-lactones, the vinyl proton of the *Z* isomer displayed a characteristic triplet at $\delta$ 6.0 in its $^1$H NMR spectra, whereas the corresponding signal of the *E* isomer appeared more downfield at $\delta = 6.7$.[18] Removal of the protecting *p*-methoxyphenyl group from each isomer with CAN was followed by acylation with pivaloyl chloride to give penultimate intermediates (*E*)-**12** and (*Z*)-**12**, which after a final deprotection with $BCl_3$ to remove the benzyl groups afforded the desired target dimethyl DAG-lactones (*E*)-**3** and (*Z*)-**3** in excellent yield.

## Biological Results and Discussion

The synthetic strategy described above essentially resulted in the transferring of two hydrophobic methyl groups of ca. 0.5 log *P* units each from the α-alkylidene side chain of high-affinity PK-C ligand **1** to the lactone core of slightly weaker DAG-lactone **2**. The intent of this exercise, leading to DAG-lactone **3**, was to minimize the nonspecific interactions involving the branched side chain with the membrane while maintaining the favorable log *P* of DAG-lactone **1**, which was indeed corroborated by the 0.12 log *P* difference between **1** and **3**.

**Table 1.** Binding Affinities ($K_i$) of (*Z*)-**3** and (*E*)-**3** for the Intact α-Isozyme and the Isolated C1b Domain of the δ-Isozyme[a]

| | (*Z*)-**3** | (*E*)-**3** | (*Z*)-**1** | (*E*)-**1** |
|---|---|---|---|---|
| PK-Cα (C1a + C1b) | 680 ± 47.3 | 1668 ± 17.2 | 2.90 ± 0.4 | 3.25 ± 0.15 |
| PK-Cδ (C1b) | 1457 ± 95.4 | 3473 ± 102.8 | 1.16 ± 0.07 | 0.90 ± 0.07 |

[a] $K_i$ values are in nM.

In addition, such transfer of lipophilicity from the side chain to the lactone core was also expected to increase specific protein−ligand interactions with the receptor, provided that the newly introduced methyl groups would not interfere with the hydrogen-bonding network involving the DAG-lactone pharmacophores. Surprisingly, the corresponding (*Z*)-**3** isomer showed a ca. 230-fold decrease in binding affinity to PK-Cα and an even higher ca. 1250-fold drop in binding affinity to the isolated C1b domain of PK-Cδ, relative to that of compound **1** (Table 1). The (*E*)-**3** isomer experienced binding reductions of ca. 500-fold and ca. 3800-fold to PK-Cα and PK-Cδ(C1b), respectively (Table 1).

Docking of either (*Z*)-**3** or (*E*)-**3** into the empty C1b domain of PK-Cδ, whose coordinates were obtained from the crystal structure of the binary complex of this domain with phorbol-13-*O*-acetate,[9] showed that the methylated lactone does not fit as well as the unsubstituted lactone into the C1 domain. The lactone ring is forced to tilt up slightly and is unable to penetrate deeply into the binding pocket because of steric clashes between the methyl groups and Pro 241 (Figure 3), and as a result, optimal distances of important hydrogen bonds in the complex are affected. Although such an unexpected steric barrier has been observed only for the two isozymes (α and δ) reported here, the highly conserved nature of this proline throughout the entire set of isozymes, except for the atypical PK-Cζ and PK-Cι/λ, which do not bind DAG, suggests this would hold true for the rest of the PK-C family.

From this result, we conclude that the binding pocket of the C1 domain of PK-C is probably narrower, sterically restricted, and more rigid than previously anticipated. Such an apparent rigidity and narrowness of the binding pocket must, therefore, be considered in the future design of new DAG-lactones. In previous work,[24] when a single hydroxyl group was introduced at C-3 of the lactone, in both stereochemical dispositions, the resulting compounds were also weaker ligands, and binding affinities dropped 10- to 200-fold. This drop in binding affinity reflected the absence of additional hydrophilic contacts between the hydroxyl groups with the receptor and the corresponding desolvation penalty incurred during binding in a lipid milieu. From this work, we have now learned that adding lipophilic methyl groups to the same position also reduces binding affinity suggesting that the C-3 position of the DAG-lactones must remain intact without substitution. This finding also highlights the structural rigidity of the first loop in the binding site (residues 238−243) perhaps due in part to the presence of the conserved proline 241 residue discussed above.

## Conclusions

It is clear from this study that geminal methyl groups at the C-3 position of the lactone hinder penetration of

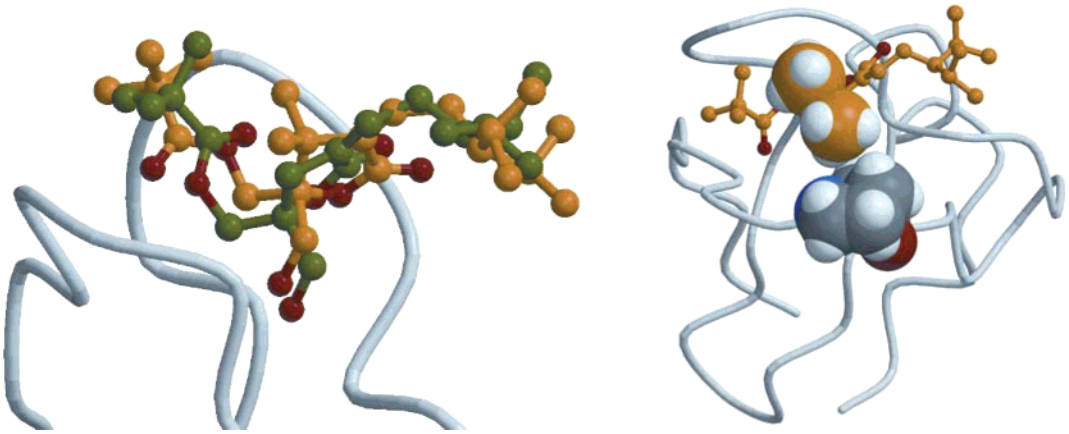


**Figure 3.** Overlapping of (*Z*)-**2** (green) and (*Z*)-**3** (yellow) showing the poorer penetration of (*Z*)-**3** due to its slightly twisted position (left) and top view showing the steric interaction between the methyl groups of (*Z*)-**3** (yellow) and Pro 241 (right).

the ligand into the active site, fail to engage in positive hydrophobic contacts with the receptor, and possibly weaken the hydrogen-bonding network of the DAG-lactone pharmacophores. It can also be concluded that the binding pocket must be quite narrow and not very flexible because, despite having an adequate lipophilicity, compounds (*Z*)-**3** and (*E*)-**3** cannot bind effectively inside the pocket. We can surmise that the C-3 position of the DAG-lactones must remain intact without substitution, and such constraints must, therefore, be considered in the future design of new DAG-lactones.

## Experimental Section

**Molecular Modeling.** The structure for the methylated lactone (compound (*Z*)-**3**) was built in Sybyl[25] and minimized with the MMFF94 force field and partial charges.[26] Docking was then performed, using FlexX[27] through its SYBYL module, into the crystal structure of the C1b domain of PK-C$\delta$.[9] The binding site was defined as residues 238−243, 250−254, and 257. The ring structure of the ligand was treated flexibly, and all other options were set to their default values. Figures were generated using MolScript[28] and Raster3D.[29]

**Analysis of Inhibition of [$^3$H]PDBU Binding by Nonradioactive Ligands and log *P*.** Enzyme−ligand interactions were analyzed by competition with [$^3$H]PDBU binding for the single isozyme PK-C$\alpha$ essentially as described previously.[18] The $ID_{50}$ values were determined by least-squares fitting of the theoretical sigmoidal competition curve to the binding data. The $K_i$ was calculated from the $ID_{50}$ values according to the relationship

$$K_i = \frac{ID_{50}}{1 + \frac{L}{K_d}}$$

where $L$ is the concentration of free [$^3$H]PDBU at the $ID_{50}$ and $K_d$ is the dissociation constant for [$^3$H]PDBU under the assay conditions.[30] Values represent the mean ± standard error (three determinations). The octanol/water partition coefficients (log *P*) were calculated according to the fragment-based program KOWWIN 1.67 (http://www.epa.gov/oppt/exposure/docs/episuitedl.htm).[21]

**General Procedures.** All chemical reagents were commercially available. AIBN = 2,2′-azobisisobutyronitrile, CAN = cerium(IV) ammonium nitrate, DMF = *N*,*N*-dimethylformamide, DMAP = dimethylamino pyridine, LDA = lithium diisopropylamide, MPM = methoxyphenylmethyl, MsCl = methanesulfonyl chloride, NBS = *N*-bromosuccinimide, NMO = 4-methylmorpholine *N*-oxide, and TPAP = tetrapropylammonium perruthenate. Column chromatography was performed on silica gel 60, 230−400 mesh (E. Merck). $^1$H and $^{13}$C NMR spectra were recorded on a Bruker AC-250 instrument at 250 and 62.9 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 Perkin-Elmer 1600 series FT-IR. Positive ion fast-atom bombardment mass spectra (FAB-MS) were obtained on a VG 7070E mass spectrometer at an accelerating voltage of 6 kV and a resolution of 2000. Glycerol was used as the sample matrix, and ionization was effected by a beam of xenon atoms. Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, Georgia.

**1-(4-Methoxyphenoxy)-3-methyl-2-[(phenylmethoxy)methyl]but-3-en-2-ol (5).** A −78 °C stirring solution of 1-(benzyloxy)-3-(4-methoxyphenoxy)acetone[23] (1.18 g, 4.1 mmol) in THF (5 mL) was treated dropwise with isopropenylmagnesium bromide. The temperature was raised to −25 °C, and the solution was stirred at this temperature for 2 h. The reaction was quenched with saturated aqueous $NH_4Cl$ and concentrated in vacuo. The residue was extracted with EtOAc, washed with water, dried over $MgSO_4$, and concentrated. Purification by flash column chromatography gave **5** (675 mg, 50%) as an oil. IR (neat) 3571 (OH), 3019 (CH), 2934 (CH), 2868 (CH), 1643 (C=C) cm$^{-1}$; $^1$H NMR ($CDCl_3$) $\delta$ 1.95 (s, 3 H, CC($CH_3$)=$CH_2$), 2.92 (br s, 1 H, CO*H*), 3.72−3.84 (app q, 2 H, PhC$H_2$OC$H_2$C), 3.86 (s, 3 H, C$H_3$O$C_6H_4$OC$H_2$C), 4.12 (s, 2 H, $CH_3OC_6H_4$-OC$H_2$C), 4.69 (s, 2 H, PhC$H_2$OC$H_2$C), 5.16 (br s, 1 H, CC($CH_3$)=C*H*H), 5.33 (br s, 1 H, CC($CH_3$)=CH*H*), 5.33−6.98 (m, 4 H, $CH_3OC_6H_4OCH_2C$), 7.34−7.45 (m, 5 H, *Ph*$CH_2$-$OCH_2C$);$^{13}$C NMR ($CDCl_3$) $\delta$ 19.65, 55.64, 71.74, 72.84, 73.55, 76.15, 112.87, 114.48, 115.66, 127.56, 127.61, 128.29, 137.79, 145.39, 152.76, 153.94; FAB-MS (*m*/*z*, relative intensity): 328 ($MH^{+\bullet}$, 39), 91 (100). Anal. ($C_{20}H_{24}O_4$) C, H.

**2-Bromo-1-methoxy-1-{1-[(4-methoxyphenoxy)methyl]-2-methyl-1-[(phenylmethoxy)methyl]prop-2-enyloxy}-ethane (6).** Under an argon atmosphere, compound **5** (679 mg, 2.07 mmol) was added to a room-temperature solution of ethylvinyl ether (1.5 mL, 15.6 mmol) in $CH_2Cl_2$ (3 mL) in a flask containing dried and activated 4-Å molecular sieves (2 g). The reaction was cooled to 0 °C, NBS (552 mg, 3.10 mmol) was added, and the reaction was stirred for 2 h. After reaching ambient temperature, the solution was filtered, and the filtrate was reduced to dryness in vacuo. Following purification by silica gel flash column chromatography, compound **6** (221 mg, 22%) was obtained as an inseparable mixture of diastereomers. IR (neat) 3020 (CH), 2979 (CH), 2935 (CH), 1642 (C=C) cm$^{-1}$; FAB-MS (*m*/*z*, relative intensity): 478 ($MH^{+\bullet}$, 30), 91 (100). Anal. ($C_{24}H_{31}BrO_5$) C, H.

**1-({5-Ethoxy-3,3-dimethyl-2-[(phenylmethoxy)methyl]-oxolan-2-yl}methoxy)-4-methoxybenzene (7).** According to a literature procedure,[22] *n*-$Bu_3SnH$ (165 $\mu$L, 0.61 mmol) was added dropwise over 30 min, under a blanket of argon, to a solution of **6** (196 mg, 0.41 mmol) and AIBN (1.34 mg, 0.008 mmol) in benzene (2 mL) at room temperature. After 4 h, the reaction mixture was heated to 45 °C for 4 h and then stirred at room temperature for 1 day. Additional *n*-$Bu_3SnH$ (200 $\mu$L,

0.75 mmol) was then added, and the reaction was again heated to 50 °C for 4 h and then stirred at room temperature for 1 day. The reaction mixture was applied directly to a silica gel flash chromatography column to give compound **7** (150 mg, 91%) as an inseparable mixture of diastereomers. IR (neat) 3019 (CH), 2963 (CH), 2934 (CH) $cm^{-1}$; FAB-MS (*m*/*z*, relative intensity): 400 ($MH^{+\bullet}$, 46), 91 (100). Anal. ($C_{24}H_{32}O_5 \cdot 0.8H_2O$) C, H.

**5-[(4-Methoxyphenoxy)methyl]-4,4-dimethyl-5-[(phenylmethoxy)methyl]oxolan-2-ol (8).** HCl (2 N, 7 mL) was added at 0 °C to a solution of **7** (222 mg, 0.55 mmol) in THF (10.5 mL) and $H_2O$ (4 mL). After 5 h at 0 °C, the reaction was allowed to reach room temperature and was stirred overnight. The reaction was quenched with solid $NaHCO_3$ followed by $H_2O$. The aqueous solution was extracted with $CH_2Cl_2$, dried, and concentrated. Purification by flash column chromatography gave **8** (140 mg, 77%) as an inseparable mixture of diastereomers. IR (neat) 3484 (OH), 3020 (CH), 2956 (CH), 2876 (CH) $cm^{-1}$; FAB-MS (*m*/*z*, relative intensity): 372 ($MH^{+\bullet}$, 7), 91 (100). Anal. ($C_{22}H_{28}O_5 \cdot 0.1H_2O$) C, H.

**5-[(4-Methoxyphenoxy)methyl]-4,4-dimethyl-5-[(phenylmethoxy)methyl]-3,4,5-trihydrofuran-2-one (9).** According to a literature procedure,[31] solid TPAP (5 mol %, 506 mg, 0.16 mmol) was added in one portion to a stirred mixture of **8** (119 mg, 0.32 mmol), NMO (60 mg, 0.48 mmol), and powdered 4-Å molecular sieves (1650 mg) in $CH_2Cl_2/CH_3CN$ (9/1, 0.65 mL) at 0 °C under argon. After 4.5 h, the reaction mixture was warmed to room temperature and stirred for 1 day. Additional NMO (35 mg) was added, and the reaction was stirred for an extra day. The crude mixture was then filtered through Celite, and the filtrate was concentrated. Purification by silica gel flash column chromatography gave **9** (78 mg, 66%) as an oil. IR (neat) 3021 (CH), 2938 (CH), 2877 (CH), 1774 (C=O) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 1.32 and 1.36 (s, 3 H, lactone-C$H_3$), 4.63 (AB quartet, $J$ = 17.2 Hz, 2 H, lactone-C$H_2$), 3.90 (AB quartet, $J$ = 10.2 Hz, 2 H, $PhCH_2OCH_2C$), 3.85 (s, 3 H, $CH_3OC_6H_4OCH_2C$), 4.23 (AB quartet, $J$ = 10.0 Hz, 2 H, $CH_3OC_6H_4OCH_2C$), 4.64 (s, 2 H, $PhCH_2OCH_2C$), 6.92 (s, 4 H, $CH_3OC_6H_4OCH_2C$), 7.34−7.47 (m, 5 H, $PhCH_2OCH_2C$);$^{13}C$ NMR ($CDCl_3$) $\delta$ 24.00, 24.11, 40.55, 45.28, 55.64, 69.82, 71.32, 73.75, 87.54, 114.58, 115.45, 127.45, 127.70, 128.34, 137.37, 152.07, 154.22, 175.74; FAB-MS (*m*/*z*, relative intensity): 370 ($M^{+\bullet}$, 98), 91 (100). Anal. ($C_{22}H_{26}O_5$) C, H.

**5-[(4-Methoxyphenoxy)methyl]-4,4-dimethyl-3-[4-methyl-3-(methylethyl)pentylidene]-5-[(phenylmethoxy)methyl]-4,5-dihydrofuran-2-one (10).** A stirred solution of **9** (514 mg, 1.39 mmol) in THF (4 mL) was treated dropwise, under a blanket of argon at −78 °C, with LDA (1.11 mL, 2 M, in heptane/THF/ethylbenzene). After stirring at −78 °C for 2 h, a solution of 3,3-diisopropylpropionaldehyde[18] in THF (1 mL) was added dropwise at the same temperature. After stirring at −78 °C for 1 day, the reaction was quenched with saturated aqueous $NH_4Cl$, and then allowed to reach ambient temperature. The aqueous layer was extracted with diethyl ether, and the extract was washed with $H_2O$, dried, and concentrated to give an oil, which was then immediately taken up in $CH_2Cl_2$ (15 mL). $Et_3N$ (774 $\mu$L, 5.5 mmol) was added, and the resulting solution was cooled to 0 °C. MsCl was then added dropwise, and the solution was stirred at the same temperature for 30 min and then at room temperature for 2 h. The reaction mixture was cooled again to 0 °C, DBU (1.04 mL, 6.94 mmol) was added, and the resulting solution was stirred at room temperature for 3 h. Concentration and purification by silica gel flash column chromatography gave a mixture of products that were immediately dissolved in DMF (0.4 mL) and treated with NaH (6 mg, 60% immersion in mineral oil) at 0 °C for 1 h. The reaction was then cooled to −78 °C, quenched with saturated aqueous $NH_4Cl$, and warmed to room temperature. The resulting mixture was extracted with diethyl ether, and the extract washed with brine, dried, and concentrated. Purification by silica gel flash column chromatography gave (*Z*)-**10** (52 mg, 26%) and (*E*)-**10** (104 mg, 13%) after four steps. (*Z*)-**10**, IR (neat) 3020 (CH), 2959 (CH), 2872 (CH), 1751 (C=O) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 0.93 and 0.95 (d, $J$ = 2.2 Hz, 3 H, C=CHCH$_2$CH(CH(C$H_3$)$_2$)$_2$), 0.97 and 1.00 (d, $J$ = 1.7 Hz, 3 H, C=CHCH$_2$CH(CH(C$H_3$)$_2$)$_2$), 1.15−1.26 (m, 1 H, C=CHCH$_2$C*H*-(CH(CH$_3$)$_2$)$_2$), 1.31 and 1.34 (s, 3 H, lactone-C$H_3$), 1.73−1.97 (m, 2 H, C=CHCH$_2$CH(C*H*(CH$_3$)$_2$)$_2$), 2.78 (d, $J$ = 5.9 Hz, 1 H, C=CHC*H*HCH(CH(CH$_3$)$_2$)$_2$), 2.81 (d, $J$ = 6.1 Hz, 1 H, C=CH-CH*H*CH(CH(CH$_3$)$_2$)$_2$), 3.79−3.89 (m, 2 H, PhCH$_2$OC$H_2$C), 3.85 (s, 3 H, C$H_3$OC$_6$H$_4$OCH$_2$C), 4.21 (AB quartet, $J$ = 9.8 Hz, 2 H, CH$_3$OC$_6$H$_4$OC$H_2$C), 4.64 (AB quartet, $J$ = 12.2 Hz, 2 H, PhC$H_2$-OCH$_2$C), 6.02 (t, $J$ = 7.3 Hz, 1 H, C=C*H*CH$_2$CH(CH(CH$_3$)$_2$)$_2$), 6.89 (s, 4 H, CH$_3$OC$_6$*H*$_4$OCH$_2$C), 7.33−7.40 (m, 5 H, *Ph*CH$_2$-OCH$_2$C);$^{13}C$ NMR ($CDCl_3$) $\delta$ 19.45, 21.70, 21.72, 23.88, 24.21, 25.81, 29.34, 29.38, 44.16, 51.35, 55.63, 68.85, 70.25, 73.60, 85.22, 114.47, 115.53, 127.35, 127.51, 128.21,134.89, 137.57, 141.77, 152.29, 154.07, 168.88; FAB-MS (*m*/*z*, relative intensity): 494 ($M^{+\bullet}$, 51), 91 (100). Anal. ($C_{31}H_{42}O_5$) C, H. (*E*)-**10**, IR (neat) 3020 (CH), 2958 (CH), 2891 (CH), 1750 (C=O) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 0.92 and 0.95 (d, $J$ = 2.0 Hz, 3 H, C=CHCH$_2$CH(CH(C$H_3$)$_2$)$_2$), 0.97 and 0.99 (br s, 3 H, C=CH-CH$_2$CH(CH(C$H_3$)$_2$)$_2$), 1.25−1.37 (m, 1 H, C=CHCH$_2$C*H*(CH-(CH$_3$)$_2$)$_2$), 1.49 and 1.51 (s, 3 H, lactone-C$H_3$), 1.82−1.92 (m, 2 H, C=CHCH$_2$CH(C*H*(CH$_3$)$_2$)$_2$), 2.38 (t, $J$ = 6.6 Hz, 2 H, C=CH-C$H_2$CH(CH(CH$_3$)$_2$)$_2$), 3.82−3.94 (m, 2 H, PhCH$_2$OC$H_2$C), 3.85 (s, 3 H, C$H_3$OC$_6$H$_4$OCH$_2$C), 4.24 (AB quartet, $J$ = 9.8 Hz, 2 H, CH$_3$OC$_6$H$_4$OC$H_2$C), 4.63 (s, 2 H, PhC$H_2$OCH$_2$C), 6.72 (t, $J$ = 7.1 Hz, 1 H, C=C*H*CH$_2$CH(CH(CH$_3$)$_2$)$_2$), 6.84−6.92 (m, 4 H, CH$_3$OC$_6$*H*$_4$OCH$_2$C), 7.35−7.42 (m, 5 H, *Ph*CH$_2$OCH$_2$C);$^{13}C$ NMR ($CDCl_3$) $\delta$ 19.50, 19.60, 21.43, 22.64, 22.76, 25.30, 28.91, 28.96, 43.72, 50.87, 55.64, 69.21, 70.58, 73.66, 85.13, 114.48, 115.38, 127.28, 127.52, 128.22, 134.83, 137.51, 140.39, 152.21, 154.06, 170.38; FAB-MS (*m*/*z*, relative intensity): 495 ($MH^+$, 49), 91 (100). Anal. ($C_{31}H_{42}O_5 \cdot 0.2H_2O$) C, H.

**(*Z*)-5-(Hydroxymethyl)-4,4-dimethyl-3-[4-methyl-3-(methylethyl)pentylidene]-5-[(phenylmethoxy)methyl]-4,5-dihydrofuran-2-one ((*Z*)-11).** According to a literature procedure,[23] ammonium cerium(IV) nitrate was added to a 0 °C solution of (*Z*)-**10** (104 mg, 0.21 mmol) in $CH_3CN/H_2O$ (4/1, 3.5 mL). After 30 min, the reaction mixture was diluted with $CH_2Cl_2$, and the layers were separated. The organic layer was washed with brine, dried, and concentrated. Purification by silica gel flash column chromatography gave (*Z*)-**11** (59 mg, 72%) as an oil. IR (neat) 3591 (OH), 3022 (CH), 2961 (CH), 2873 (CH), 1750 (C=O), 1664 (C=C) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 0.91, 0.94, 0.96 and 0.99 (s, 3 H, C=CHCH$_2$CH(CH(C$H_3$)$_2$)$_2$), 1.12−1.22 (m, 1 H, C=CHCH$_2$C*H*(CH(CH$_3$)$_2$)$_2$), 1.25 and 1.27 (s, 3 H, lactone-C$H_3$), 1.78−1.91 (m, 2 H, C=CHCH$_2$CH-(C*H*(CH$_3$)$_2$)$_2$), 2.42 (br s, 1 H, *H*OCH$_2$C), 2.68−2.79 (m, 2 H, C=CHC$H_2$CH(CH(CH$_3$)$_2$)$_2$), 3.71−3.93 (overlapping AB quartets, $J$ = 12.0 and 9.7 Hz, 4 H, C$_6$H$_5$CH$_2$OC$H_2$C and HOC$H_2$C), 4.62 (AB quartet, $J$ = 12.2 Hz, 2 H, C$_6$H$_5$CH$_2$OC$H_2$C), 6.03 (t, $J$ = 7.3 Hz, 1 H, C=C*H*CH$_2$CH(CH(CH$_3$)$_2$)$_2$), 7.34−7.42 (m, 5 H, *Ph*CH$_2$OCH$_2$C);$^{13}C$ NMR ($CDCl_3$) $\delta$ 19.37, 19.40, 21.69, 21.72, 22.82, 24.41, 25.85, 29.36, 29.39, 44.01, 51.32, 63.41, 69.42, 73.69, 127.45, 127.65, 128.28, 134.47, 137.42, 142.78, 169.04; FAB-MS (*m*/*z*, relative intensity): 389 ($MH^+$, 40), 91 (100). Anal. ($C_{24}H_{36}O_4 \cdot 0.5H_2O$) C, H.

**(*E*)-5-(Hydroxymethyl)-4,4-dimethyl-3-[4-methyl-3-(methylethyl)pentylidene]-5-[(phenylmethoxy)methyl]-4,5-dihydrofuran-2-one ((*E*)-11).** According to the same literature procedure as used for (*Z*)-**11**,[23] ammonium cerium(IV) nitrate was added to a 0 °C solution of (*E*)-**10** (52 mg, 0.11 mmol) in $CH_3CN/H_2O$ (4/1, 1.8 mL) to give (*E*)-**11** (23 mg, 57%) as an oil. IR (neat) 3692 (OH), 3020 (CH), 2959 (CH), 2870 (CH), 1734 (C=O), 1664 (C=C) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 0.92 and 0.95 (d, $J$ = 1.2 Hz, 3 H, C=CHCH$_2$CH(CH(C$H_3$)$_2$)$_2$), 0.97 and 0.99 (d, $J$ = 3.4 Hz, 3 H, C=CHCH$_2$CH(CH(C$H_3$)$_2$)$_2$), 1.24−1.34 (m, 1 H, C=CHCH$_2$C*H*(CH(CH$_3$)$_2$)$_2$), 1.42 and 1.48 (s, 3 H, lactone-C$H_3$), 1.80−1.92 (m, 2 H, C=CHCH$_2$CH(C*H*(CH$_3$)$_2$)$_2$), 2.13 (br s, 1 H, *H*OCH$_2$C), 2.34−2.39 (irregular t, 2 H, C=CH-C$H_2$CH(CH(CH$_3$)$_2$)$_2$), 3.82 (AB quartet, $J$ = 10.0 Hz, 4 H, C$_6$H$_5$-CH$_2$OC$H_2$C), 3.92 (s, 2 H, HOC$H_2$C), 4.62 (AB quartet, $J$ = 12.0 Hz, 2 H, C$_6$H$_5$C$H_2$OCH$_2$C), 6.72 (t, 1 H, $J$ = 6.9 Hz, C=C-*H*CH$_2$CH(CH(CH$_3$)$_2$)$_2$), 7.36−7.42 (m, 5 H, *Ph*CH$_2$O-CH$_2$C);$^{13}C$ NMR ($CDCl_3$) $\delta$ 19.48, 19.54, 21.36, 21.50, 22.03, 22.79, 25.40, 28.93, 28.99, 43.62, 50.88, 63.55, 69.49, 73.74,

86.09, 127.37, 127.64, 128.27, 134.26, 137.42, 141.99, 170.41; FAB-MS (*m*/*z*, relative intensity): 389 ($MH^+$, 91), 91 (100). Anal. ($C_{24}H_{36}O_4 \cdot 0.2H_2O$) C, H.

**(*Z*)-{3,3-Dimethyl-4-[4-methyl-3-(methylethyl)pentylidene]-5-oxo-2-[(phenylmethoxy)methyl]-2−2,3-dihydrofuryl}methyl-2,2-dimethylpropanoate ((*Z*)-12).** Under a blanket of argon, trimethylacetyl chloride (59 $\mu$L, 0.0.48 mmol) was added to a 0 °C solution of (*Z*)-**11** (460 mg, 0.12 mmol) and DMAP (5 mg, 0.041 mmol) in pyridine (539 $\mu$L). The resulting solution was allowed to warm to room temperature over 7 h and then was concentrated. Purification by silica gel flash column chromatography gave (*Z*)-**12** (50 mg, 88%) as an oil. IR (neat) 3020 (CH), 2962 (CH), 2872 (CH), 1752 (C═O), 1665 (C═C) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 0.92 and 0.94 (s, 3 H, C═CHCH$_2$CH(CH(C$H_3$)$_2$)$_2$), 0.97 (d, $J$ = 4.4 Hz, 3 H, C═CHCH$_2$CH(CH(C$H_3$)$_2$)$_2$), 0.99 (d, $J$ = 4.6 Hz, 3 H, C═CHCH$_2$CH(CH(C$H_3$)$_2$)$_2$), 1.24 (s, 9 H, (C$H_3$)$_3$CO$_2$CH$_2$C), 1.27 (br s, 6 H, lactone-C$H_3$), 1.78−1.94 (m, 2 H, C═CHCH$_2$CH(C$H$(CH$_3$)$_2$)$_2$), 2.51−2.62 (m, 1 H, C═CHC$H$HCH(CH(CH$_3$)$_2$)$_2$), 2.91−3.03 (m, 1 H, C═CHCH$H$CH(CH(CH$_3$)$_2$)$_2$), 3.72 (AB quartet, $J$ = 9.5 Hz, 2 H, PhCH$_2$OC$H_2$C), 4.35 (AB quartet, $J$ = 12.2 Hz, 2 H, (CH$_3$)$_3$CO$_2$C$H_2$C), 4.59 (s, 2 H, PhC$H_2$OCH$_2$C), 6.02 (dd, 1 H, $J$ = 8.3, 6.4 Hz, C═C$H$CH$_2$CH(CH(CH$_3$)$_2$)$_2$), 7.34−7.41 (m, 5 H, $Ph$CH$_2$OCH$_2$C);$^{13}C$ NMR ($CDCl_3$) $\delta$ 19.22, 19.58, 21.57, 21.79, 23.45, 24.54, 25.77, 26.99, 29.24, 29.45, 38.69, 44.03, 51.31, 64.49, 69.72, 73.65, 84.52, 127.42, 127.65, 128.26, 134.54, 137.27, 142.33, 168.64, 177.60; FAB-MS (*m*/*z*, relative intensity): 473 ($MH^+$, 28), 91 (100). Anal. ($C_{29}H_{44}O_5$) C, H.

**(*E*)-{3,3-Dimethyl-4-[4-methyl-3-(methylethyl)pentylidene]-5-oxo-2-[(phenylmethoxy)methyl]-2−2,3-dihydrofuryl}methyl-2,2-dimethylpropanoate ((*E*)-12).** Following the same procedure as that used for (*Z*)-**12**, (*E*)-**11** (20 mg, 0.05 mmol) was converted to give (*E*)-**12** (23 mg, 92%) as an oil. IR (neat) 3020 (CH), 2962 (CH), 2874 (CH), 1731 (C═O), 1665 (C═C) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 0.92 and 0.95 (s, 3 H, C═CHCH$_2$CH(CH(C$H_3$)$_2$)$_2$), 0.96 and 0.99 (d, $J$ = 4.4 Hz, 3 H, C═CHCH$_2$CH(CH(C$H_3$)$_2$)$_2$), 1.24 (s, 9 H, (C$H_3$)$_3$CO$_2$CH$_2$C), 1.26−133 (m, 1 H, C═CHCH$_2$C$H$(CH(CH$_3$)$_2$)$_2$), 1.43 (s, 3 H, lactone-C$H_3$), 1.49 (s, 3 H, lactone-C$H_3$), 1.83−1.93 (m, 2 H, C═CHCH$_2$CH(C$H$(CH$_3$)$_2$)$_2$), 2.34−2.39 (m, 2 H, C═CHC$H_2$CH(CH(CH$_3$)$_2$)$_2$), 3.77 (s, 2 H, PhCH$_2$OC$H_2$C), 4.35 (AB quartet, $J$ = 12.2 Hz, 2 H, (CH$_3$)$_3$CO$_2$C$H_2$C), 4.60 (s, 2 H, PhC$H_2$-OCH$_2$C), 6.72 (irregular t, 1 H, $J$ = 6.9 Hz, C═C$H$CH$_2$CH(CH(CH$_3$)$_2$)$_2$), 7.34−7.45 (m, 5 H, $Ph$CH$_2$OCH$_2$C); $^{13}C$ NMR ($CDCl_3$) $\delta$ 19.32, 19.68, 21.27, 21.45, 22.45, 22.82, 25.31, 26.94, 28.79, 28.97, 38.68, 43.55, 50.77, 65.15, 70.18, 73.73, 84.49, 12.737, 127.66, 128.27, 134.60, 137.20, 140.98, 170.04, 177.61; FAB-MS (*m*/*z*, relative intensity): 473 ($MH^+$, 45), 91 (100). Anal. ($C_{29}H_{44}O_5$) C, H.

**(*Z*)-{2-(Hydroxymethyl)-3,3-dimethyl-4-[4-methyl-3-(methylethyl)pentylidene]-5-oxo-2−2,3-dihydrofuryl}methyl 2,2-dimethylpropanoate ((*Z*)-3).** A solution of $BCl_3$ (0.17 mL, 1 M in $CH_2Cl_2$) was added slowly to a −78 °C solution of (*Z*)-**12** (39.6 mg, 0.09 mmol) in $CH_2Cl_2$ (1.5 mL) and was stirred for 2 h at the same temperature. The buffer solution (pH 7.2, 1 mL) was slowly added at −78 °C, and then the mixture was diluted with diethyl ether. The layers were separated, and the aqueous layer was extracted with $CHCl_3$ (5 mL), dried, and concentrated. Purification by silica gel (neutralized with 2% $Et_3N$/hexane) flash column chromatography gave (*Z*)-**3** (29.5 mg, 92%) as an oil. IR (neat) 3587 (OH), 3020 (CH), 2962 (CH), 2874 (CH), 1752 (C═O), 1664 (C═C) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 0.92 and 0.95 (s, 3 H, C═CHCH$_2$-CH(CH(C$H_3$)$_2$)$_2$), 0.97 and 1.00 (d, $J$ = 3.2 Hz, 3 H, C═CHCH$_2$-CH(CH(C$H_3$)$_2$)$_2$), 1.12−1.25 (m, 1 H, C═CHCH$_2$C$H$(CH-(CH$_3$)$_2$)$_2$), 1.27 (s, 3 H, lactone-C$H_3$), 1.28 (s, 9 H, (C$H_3$)$_3$CO$_2$-CH$_2$C), 1.31 (s, 3 H, lactone-C$H_3$), 1.78−1.90 (m, 2 H, C═CHCH$_2$CH(C$H$(CH$_3$)$_2$)$_2$), 2.23 (br s, 1 H, $H$OCH$_2$C), 2.57−2.68 (m, 1 H, C═CHC$H$HCH(CH(CH$_3$)$_2$)$_2$), 2.86−2.98 (m, 1 H, C═CHCH$H$CH(CH(CH$_3$)$_2$)$_2$), 3.82 (s, 2 H, HOC$H_2$C), 4.38 (s, 2 H, (CH$_3$)$_3$CO$_2$C$H_2$C), 6.05−6.11 (m, 1 H, C═C$H$CH$_2$CH(CH-(CH$_3$)$_2$)$_2$);$^{13}C$ NMR ($CDCl_3$) $\delta$ 19.26, 19.51, 21.60, 21.76, 23.50, 24.19, 25.89, 27.04, 29.29, 29.44, 38.76, 43.86, 51.33, 62.42, 62.99, 85.31, 134.09, 143.42, 168.50, 177.98; FAB-MS (*m*/*z*, relative intensity): 383 ($MH^+$, 73), 57 (100). Anal. ($C_{22}H_{38}O_5 \cdot 0.1H_2O$) C, H.

**(*E*)-{2-(hydroxymethyl)-3,3-dimethyl-4-[4-methyl-3-(methylethyl)pentylidene]-5-oxo-2−2,3-dihydrofuryl}methyl 2,2-dimethylpropanoate ((*E*)-3).** A solution of $BCl_3$ (0.08 mL, 1 M in $CH_2Cl_2$) was added slowly to a −78 °C solution of (*E*)-**12** (20.4 mg, 0.04 mmol) and was stirred for 2 h at the same temperature. Following a similar work up as that for (*Z*)-**3**, compound (*E*)-**3** (15.3 mg, 93%) was obtained as an oil. IR (neat) 3589 (OH), 3020 (CH), 2962 (CH), 2875 (CH), 1751 (C═O), 1664 (C═C) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 0.93 and 0.96 (d, $J$ = 3.2 Hz, 3 H, C═CHCH$_2$CH(CH(C$H_3$)$_2$)$_2$), 0.98 and 1.01 (s, 3 H, C═CHCH$_2$CH(CH(C$H_3$)$_2$)$_2$), 1.28 (s, 9 H, (C$H_3$)$_3$CO$_2$CH$_2$C), 1.30−1.36 (m, 1 H, C═CHCH$_2$C$H$(CH-(CH$_3$)$_2$)$_2$), 1.46 and 1.48 (s, 3 H, lactone-C$H_3$), 1.85−2.10 (m, 2 H, C═CHCH$_2$CH(C$H$(CH$_3$)$_2$)$_2$), 2.10 (br s, 1 H, $H$OCH$_2$C), 2.35−2.41 (m, 2 H, C═CHC$H_2$CH(CH(CH$_3$)$_2$)$_2$), 3.89 (s, 2 H, HOC$H_2$C), 4.41 (AB quartet, $J$ = 12.1 Hz, 2 H, (CH$_3$)$_3$-CO$_2$C$H_2$C), 6.76 (irregular t, $J$ = 7.0 Hz, 1 H, C═C$H$CH$_2$CH-(CH(CH$_3$)$_2$)$_2$);$^{13}C$ NMR ($CDCl_3$) $\delta$ 19.41, 19.60, 21.38, 21.41, 22.45, 22.72, 25.44, 27.00, 28.89, 28.97, 38.73, 43.43, 50.84, 62.60, 63.60, 85.29, 134.08, 142.30, 169.96, 177.92; FAB-MS (*m*/*z*, relative intensity): 383 ($MH^+$, 77), 57 (100). Anal. ($C_{22}H_{38}O_5$) C, H.

**Supporting Information Available:** Elemental analysis data for **5**−**10**, **(*E*)-** and **(*Z*)-3**, **11**, **12**, and **(*E*)-10**. This material is available free of charge via the Internet at http://pubs.acs.org.

## References


(1) Castagna, M.; Takai, Y.; Kaibuchi, K.; Sano, K.; Kikkawa, U.; Nishizuka, Y. Direct activation of calcium-activated, phospholipid-dependent protein kinase by tumor-promoting phorbol esters. *J. Biol. Chem.* **1982**, *257*, 7847−7851.

(2) Newton, A. C. Protein kinase C: Structural and spatial regulation by phosphorylation, cofactors, and macromolecular interactions. *Chem. Rev.* **2001**, *101*, 2353−2364.

(3) Newton, A. C.; Keranen, L. M. Phosphatidyl-L-serine is necessary for protein-kinase Cs high-affinity interaction with diacylglycerol-containing membranes. *Biochemistry* **1994**, *33*, 6651−6658.

(4) Newton, A. C. Regulation of protein kinase C. *Curr. Opin. Cell Biol.* **1997**, *9*, 161−167.

(5) Nishizuka, Y. Intracellular signaling by hydrolysis of phospholipids and activation of protein-kinase-C. *Science* **1992**, *258*, 607−614.

(6) Oancea, E.; Teruel, M. N.; Quest, A. F. G.; Meyer, T. Green fluorescent protein (GFP)-tagged cysteine-rich domains from protein kinase C as fluorescent indicators for diacylglycerol signaling in living cells. *J. Cell Biol.* **1998**, *140*, 485−498.

(7) Sakai, N.; Sasaki, K.; Ikegaki, N.; Shirai, Y.; Ono, Y.; Saito, N. Direct visualization of the translocation of the gamma-subspecies of protein kinase C in living cells using fusion proteins with green fluorescent protein. *J. Cell Biol.* **1997**, *139*, 1465−1476.

(8) Oancea, E.; Meyer, T. Protein kinase C as a molecular machine for decoding calcium and diacylglycerol signals. *Cell* **1998**, *95*, 307−318.

(9) Zhang, G. G.; Kazanietz, M. G.; Blumberg, P. M.; Hurley, J. H. Crystal-structure of the Cys2 activator-binding domain of protein-kinase C-delta in complex with phorbol ester. *Cell* **1995**, *81*, 917−924.

(10) Van Duuren, B. L.; Tseng, S.-S.; Segal, A.; Smith, A. C.; Melchionne, S.; Seidman, I. Effects of structural changes on the tumor-promoting activity of phorbol myristate acetate on mouse skin. *Cancer Res.* **1979**, *39*, 2644−2646.

(11) Baird, W. M.; Boutwell, R. K. Tumor-promoting activity of phorbol and four diesters of phorbol in mouse skin. *Cancer Res.* **1971**, *31*, 1074−1079.

(12) El-Mekkawy, S.; Meselhy, M. R.; Nakamura, N.; Hattori, M.; Kawahata, T.; Otake, T. 12-*O*-acetylphorbol-13-decanoate potently inhibits cytopathic effects of human immunodeficiency virus type 1 (HIV-1), without activation of protein kinase C. *Chem. Pharm. Bull.* **1999**, *47*, 1346−1347.

(13) Krauter, G.; VonderLieth, C. W.; Schmidt, R.; Hecker, E. Structure/activity relationships of polyfunctional diterpenes of the tigliane type−A pharmacophore model for protein-kinase-C activators based on structure/activity studies and molecular modeling of the tumor promoters 12-O− tetradecanoylphorbol 13-acetate and 3-O-tetradecanoylingenol. *Eur. J. Biochem.* **1996**, *242*, 417−427.

(14) Kazanietz, M. G.; Krausz, K. W.; Blumberg, P. M. Differential irreversible insertion of protein kinase C into phospholipid vesicles by phorbol esters and related activators. *J. Biol. Chem.* **1992**, *267*, 20878−20886.
(15) Sharkey, N. A.; Blumberg, P. M. Comparison of the activity of phorbol 12-myristate 13-acetate and the diglyceride glycerol 1-myristate 2-acetate. *Carcinogenesis* **1986**, *7*, 677−679.
(16) Marquez, V. E.; Nacro, K.; Benzaria, S.; Lee, J.; Sharma, R.; Teng, K.; Milne, G. W.; Bienfait, B.; Wang, S.; Lewin, N. E.; Blumberg, P. M. The transition from a pharmacophore-guided approach to a receptor-guided approach in the design of potent protein kinase C ligands. *Pharmacol. Ther.* **1999**, *82*, 251−261.
(17) Marquez, V. E.; Blumberg, P. M. Synthetic diacylglycerols (DAG) and DAG-lactones as activators of protein kinase C (PK-C). *Acc. Chem. Res.* **2003**, *36*, 434−443.
(18) Nacro, K.; Bienfait, B.; Lee, J.; Han, K. C.; Kang, J. H.; Benzaria, S.; Lewin, N. E.; Bhattacharyya, D. K.; Blumberg, P. M.; Marquez, V. E. Conformationally constrained analogues of diacylglycerol (DAG). 16. How much structural complexity is necessary for recognition and high binding affinity to protein kinase C? *J. Med. Chem.* **2000**, *43*, 921−944.
(19) Nacro, K.; Bienfait, B.; Lewin, N. E.; Blumberg, P. M.; Marquez, V. E. Diacylglycerols with lipophilically equivalent branched acyl chains display high affinity for protein kinase C (PK-C). A direct measure of the effect of constraining the glycerol backbone in DAG lactones. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 653−655.
(20) Meylan, W.; Howard, P. Fragment Contribution Method for Estimating Octanol−Water Partition Coefficients. *J. Pharm. Sci.* **1995**, *84*, 83−92.
(21) Meylan, W.; Howard, P. KOWWIN 1.63; Syracuse Research Corp, 1995.
(22) Ueno, Y.; Moriya, O.; Chino, K.; Watanabe, M.; Okawara, M. General synthetic route to gamma-butyrolactones via stereoselective radical cyclization by organotin species. *J. Chem. Soc., Perkin Trans. 1* **1986**, 1351−1356.
(23) Lee, J.; Kang, J. H.; Lee, S. Y.; Han, K. C.; Torres, C. M.; Bhattacharyya, D. K.; Blumberg, P. M.; Marquez, V. E. Protein kinase C ligands based on tetrahydrofuran templates containing a new set of phorbol ester pharmacophores. *J. Med. Chem.* **1999**, *42*, 4129−4139.
(24) Nacro, K.; Lee, J.; Barchi, J. J.; Lewin, N. E.; Blumberg, P. M.; Marquez, V. E. Conformationally constrained analogues of diacylglycerol (DAG). Part 19: Asymmetric syntheses of (3*R*)- and (3*S*)-3-hydroxy-4,4-disubstituted heptono-1,4-lactones as protein kinase C (PK-C) ligands with increased hydrophilicity. *Tetrahedron* **2002**, *58*, 5335−5345.
(25) Tripos, Inc. (www.tripos.com).
(26) Halgren, T. A. Merck molecular force field. 1. Basis, form, scope, parametrization, and performance of MMFF94. *J. Comput. Chem.* **1996**, *17*, 490−519.
(27) Rarey, M.; Kramer, B.; Lengauer, T.; Klebe, G. A fast flexible docking method using an incremental construction algorithm. *J. Mol. Biol.* **1996**, *261*, 470−489.
(28) Kraulis, P. J. Molscript−a program to produce both detailed and schematic plots of protein structures. *J. Appl. Crystallogr.* **1991**, *24*, 946−950.
(29) Merritt, E. A.; Bacon, D. J. Raster3D: Photorealistic molecular graphics. *Methods Enzymol.* **1997**, *277*, 502−524.
(30) Lewin, N. E.; Blumberg, P. M. [3*H*]-Phorbol 12,13-dibutyrate binding assay for protein kinace C and related proteins. *Methods Mol. Biol.* **2003**, *233*, 129−156.
(31) Ley, S. V.; Norman, J.; Griffith, W. P.; Marsden, S. P. Tetrapropylammonium perruthenate, Pr4n+Ruo4-, Tpap−a catalytic oxidant for organic-synthesis. *Synthesis* **1994**, 639−666.

JM049723+