Conformationally Constrained Analogues of Diacylglycerol (DAG). 16.[1] How Much Structural Complexity Is Necessary for Recognition and High Binding Affinity to Protein Kinase C?

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Received September 9, 1999

The design of potent protein kinase C (PK-C) ligands with low nanomolar binding affinities was accomplished by the combined use of pharmacophore- and receptor-guided approaches based on the structure of the physiological enzyme activator, diacylglycerol (DAG). Earlier use of the former approach, which was based on the structural equivalence of DAG and phorbol ester pharmacophores, identified a fixed template for the construction of a semirigid "recognition domain" that contained the three principal pharmacophores of DAG constrained into a lactone ring (DAG-lactones). In the present work, the pharmacophore-guided approach was refined to a higher level based on the X-ray structure of the C1b domain of PK-Cδ complexed with phorbol-13-*O*-acetate. A systematic search that involved modifying the DAG-lactone template with a combination of linear or branched acyl and α-alkylidene chains, which functioned as variable hydrophobic "affinity domains", helped identify compounds that optimized hydrophobic contacts with a group of conserved hydrophobic amino acids located on the top half of the C1 domain where the phorbol binds. The hydrophilic/hydrophobic balance of the molecules was estimated by the octanol/water partition coefficients (log *P*) calculated according to a fragment-based approach. The presence of branched α-alkylidene or acyl chains was of critical importance to reach low nanomolar binding affinities for PK-C. These branched chains appear to facilitate important van der Waals contacts with hydrophobic segments of the protein and help promote the activation of PK-C through critical membrane interactions. Molecular modeling of these DAG-lactones into an empty C1b domain using the program AutoDock 2.4 suggests the existence of competing binding modes (*sn*-1 and *sn*-2) depending on which carbonyl is directly involved in binding to the protein. Inhibition of epidermal growth factor (EGF) binding, an indirect PK-C mediated response, was realized with some DAG-lactones at a dose 10-fold higher than with the standard phorbol-12,13-dibutyrate (PDBU). Through the National Cancer Institute (NCI) 60-cell line in vitro screen, DAG-lactone **31** was identified as a very selective and potent antitumor agent. The NCI's computerized, pattern-recognition program COMPARE, which analyzes the degree of similarity of mean-graph profiles produced by the screen, corroborated our principles of drug design by matching the profile of compound **31** with that of the non-tumor-promoting antitumor phorbol ester, prostratin. The structural simplicity and the degree of potency achieved with some of the DAG-lactones described here should dispel the myth that chemical complexity and pharmacological activity go hand in hand. Even as a racemate, DAG-lactone **31** showed low namomolar binding affinity for PK-C and displayed selective antitumor activity at equivalent nanomolar levels. Our present approach should facilitate the generation of multiple libraries of structurally similar DAG-lactones to help exploit molecular diversity for PK-C and other high-affinity receptors for DAG and the phorbol esters. The success of this work suggests that substantially simpler, high-affinity structures could be identified to function as surrogates of other complex natural products.

Introduction

Protein kinase C (PK-C) is a family of at least 11 serine/threonine-specific isozymes that, depending on the specific isozyme, are regulated by phosphorylation, by calcium, and by association with phospholipids and diacylglycerol (DAG).[2,3] Typically, the isozymes are cytosolic in the inactive state and, as part of the activation process, translocate to the inner leaflet of the cellular membrane.[4] Individual cell types contain different combinations of PK-C isozymes, suggesting distinct roles for these isozymes in signaling pathways that regulate cell cycle progression, differentiation, and apoptosis.[2] The binding of PK-C to the plasma membrane is transient and regulated by the association with diacylglycerol (DAG) in the membrane.[5] DAG (Figure

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10.1021/jm9904607 CCC: $19.00
Published on Web 02/23/2000

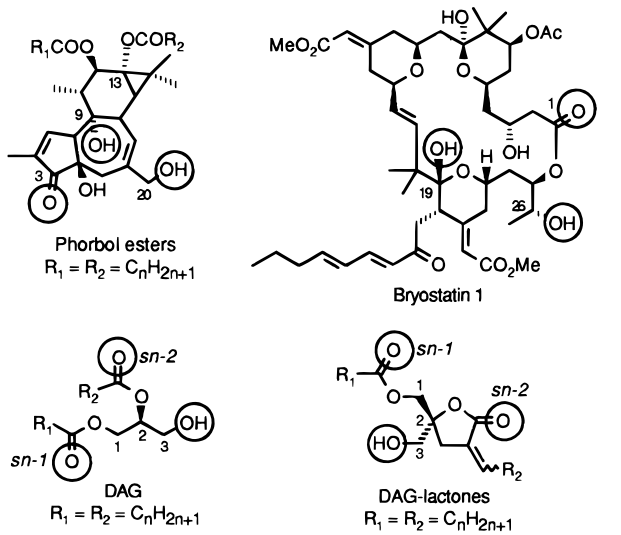


Figure 1. Chemical structures of phorbol esters, bryostatin 1, DAG, and DAG-lactones showing the critical pharmacophores suggested by computational and structure−activity studies.[11]

1) functions as a central lipophilic second messenger that is generated in response to activation of numerous receptors which are coupled either through G-protein or tyrosine kinase mechanisms to activation of phospholipase C hydrolysis of phosphatidylinositol 4,5-biphosphate.[6] DAG can also arise indirectly from phosphatidic acid produced by phospholipase D.[7] Physiologically, DAG binds to the C1 domain in members of the classical (α, *β*, and *γ*), as well as the novel (*δ*, *ϵ*, *η*, and *θ*), PK-C isozymes to activate their downstream pathways.[8] Among pharmacological agents, the phorbol esters (Figure 1) bind directly to the same C1 domain and function as potent and metabolically stable DAG surrogates.[9] Indeed, the phorbol esters bind to PK-C with affinities that are 3 to 4 orders of magnitude greater than that of DAG.[10]

Highlighting the important biological role of PK-C, several other structural classes of natural products, among them bryostatin 1 (Figure 1), have also been characterized as potent DAG analogues.[12] From studies with these natural products, it is clear that different specific ligands with similar pharmacophores that recognize PK-C (Figure 1) can differentially activate PK-C subpathways and thereby induce different patterns of biological responses. Thus, whereas the archetypical phorbol-12-myristate-13-acetate (PMA) acts as a potent tumor promoter,[13] the macrocyclic lactone bryostatin 1, currently in clinical trials as a cancer chemotherapeutic agent, is a partial functional antagonist of PMA.[14] The structural complexity of these and other natural products has prompted several groups to synthesize simpler synthetic analogues, some of which displayed significant binding affinities toward PK-C.[15−22] However, a marked degree of structural complexity still remains in these molecules, making it difficult to exploit chemical diversity to the fullest extent. Thus, the availability of only a limited number of distinct PK-C ligands represents an obstacle to understand the structural basis for their interactions with PK-C, as well as the origins of their distinct biological effects.

The stark contrast between the structurally complex natural products and the simple DAG structure (Figure 1) poses the intriguing question of whether the intricate structure of these natural products and their biological potency go hand-in-hand. To address this issue, we have sought to design synthetically accessible ligands for the C1 domain of PK-C based on the simpler DAG structure. Initially, using a pharmacophore-guided approach that was based on the geometry of bioequivalent pharmacophores present in DAG and in the phorbol esters,[11,12,23] we synthesized a number of potent DAG-lactones in which the glycerol backbone was constrained to a lactone ring to reduce the entropic penalty associated with DAG binding (Figure 1).[24−26] In the present investigation, the information provided by the X-ray[27] and NMR[28] structures of the cysteine-rich, C1 phorbol ester binding domain has been used to further modify these DAG-lactones to optimize their interaction with a group of highly conserved hydrophobic amino acids along the rim of the C1 domain.[27,28] With this approach, we sought to transform these structurally simple DAG-lactones into ligands with affinities approaching those of the phorbol esters. These compounds were intended to serve both as probes to test our understanding of the nature of ligand−C1 domain interactions and to explore the basis for biological selectivity of PK-C-targeted ligands with a library of easily obtainable DAG mimetics.

The use of the receptor-guided approach described here culminated with the synthesis of a series of molecules that bind to PK-C with affinities in the low nanomolar range. These compounds provide insight into the basis for PK-C ligand specificity, and some of them show potential utility as antitumor agents. Here, we present a discussion based on this effective drug-design strategy, report on the chemical syntheses of the new targets, and analyze the biological profile of the new potent ligands in terms of several important parameters, including binding affinity (K_i) to PK-Cα, activation of PK-Cα, inhibition of epidermal growth factor (EGF) binding as an indirect PK-C mediated response,[29] and antitumor activity in the National Cancer Institute 60-cell line in vitro screen.[30]

The Receptor-Guided Approach

A. Hydrogen Bonding. The recent X-ray structure of phorbol-13-*O*-acetate complexed to the C1b domain of PK-C*δ*[27] confirmed the importance of hydrogen bonds involving the key pharmacophores C-20 (OH) and C-3 (C═O), the latter in combination with C-4 (OH), as essential elements for the recognition of PK-C*δ* and other PK-C isozymes by the phorbol esters (see Figure 1 for phorbol's numbering). However, the presumed critical C-9 (OH) pharmacophore did not participate in any hydrogen bond to the protein and appeared instead intramolecularly hydrogen-bonded to the C-13 (C═O) ester. Since the role of the C-13 (C═O) has been shown to be critical in recent structure−activity studies,[31,32] it is possible that the intramolecular hydrogen-bonding motif involving C-9 (OH) and C-13 (C═O) binds outside the C1b domain, perhaps at the membrane−protein interface, which would explain why it appears missing in the phorbol-13-*O*-acetate/C1b complex.[27] In a recent study, we reported on the use of the program AutoDock to anchor a DAG-lactone into an empty C1b domain constructed with the same crystal coordinates as those

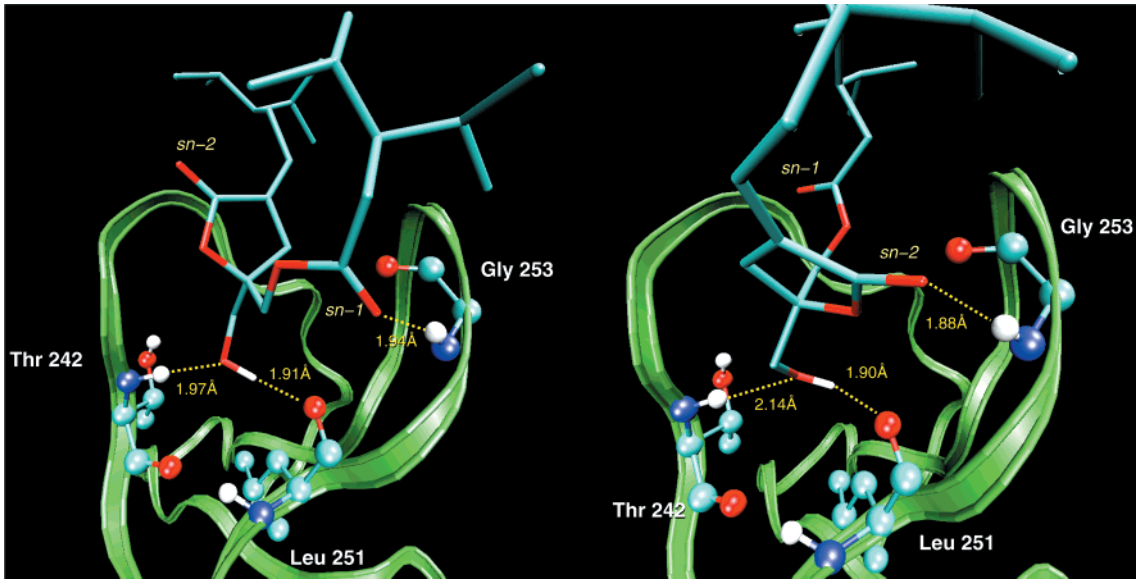
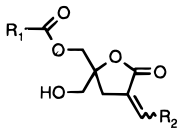


Figure 2. Docking results showing hydrogen-bonding interactions of **31** with the C1b domain of PK-Cδ in binding modes *sn*-1 (left) and *sn*-2 (right).[33]

Table 1. PK-Cα Binding Affinity (K_i) and Calculated log *P* for α-Alkylidene and Acyl Branched Lactones (*Z*- and *E*-Isomers)

	Cpd. #	*Z*-isomer K_i (binding) nM	Cpd. #	*E*-isomer K_i (binding) nM	Calcd. log P octanol/water
$R_1 = CH_3$ $R_2 = CH{=}C(i\text{-}Pr)_2$	**7**	390 ±26	**8**	409 ±26	2.59
$R_1 = CH_3(CH_2)_2$ $R_2 = CH{=}C(i\text{-}Pr)_2$	**9**	68 ±6.0	**10**	51 ±8.0	3.57
$R = CH_3(CH_2)_4$ $R_2 = CH{=}C(i\text{-}Pr)_2$	**11**	12 ±0.5	**12**	8.4 ±0.6	4.56
$R_1 = CH_3$ $R_2 = CH_2CH(i\text{-}Pr)_2$	**13**	123 ±4.5	**14**	359 ±32	2.68
$R_1 = CH_3(CH_2)_2$ $R_2 = CH_2CH(i\text{-}Pr)_2$	**15**	19 ±1.5	**16**	54 ±4.0	3.66
$R_1 = CH_3(CH_2)_4$ $R_2 = CH_2CH(i\text{-}Pr)_2$	**17**	5.9 ±0.2	**18**	11 ±0.6	4.64
$R_1 = CH_3(CH_2)_6$ $R_2 = CH_2CH(i\text{-}Pr)_2$	**19**	3.5 ±0.3	**20**	5.9 ±0.5	5.62
$R_1 = CH_3(CH_2)_8$ $R_2 = CH_2CH(i\text{-}Pr)_2$	**21**	2.3 ±0.1	**22**	6.6 ±1.0	6.61
$R_1 = CH_2CH(i\text{-}Pr)_2$ $R_2 = CH_3$	**23**	164 ±2	**24**	138 ±32	2.68
$R_1 = CH_2CH(i\text{-}Pr)_2$ $R_2 = CH_3(CH_2)_2$	**25**	24 ±1.0	**26**	28 ±6	3.66
$R_1 = CH_2CH(i\text{-}Pr)_2$ $R_2 = CH_3(CH_2)_4$	**27**	7.3 ±0.5	**28**	5.5 ±0.6	4.64
$R_1 = CH_2CH(i\text{-}Pr)_2$ $R_2 = CH_3(CH_2)_8$	**29**	13 ±0.4	**30**	9.5 ±0.9	6.61
$R_1 = R_2 = CH_2CH(i\text{-}Pr)_2$	**31**	2.9 ±0.2	**32**	2.7 ±0.4	5.89

of the C1b domain complexed with phorbol-13-*O*-acetate.[1] The program consistently identified two energetically similar binding modes with an identical network of hydrogen bonds to the same amino acids as in the phorbol-13-*O*-acetate/C1b complex. As shown in Figure 2 for DAG-lactone **31** (Table 1), these two binding modes are designated as *sn*-1 and *sn*-2 (Figure 1) depending on which of the nonequivalent carbonyl functions appears directly involved in binding to the protein. In these two binding alternatives, it is possible that the uninvolved C=O group would bind at the membrane−protein interface and be functionally equivalent to the intramolecularly hydrogen-bonded C-9 (OH)/C-13 (C=O) array of the phorbol esters. The absolute necessity for both *sn*-1 and *sn*-2 carbonyls to be simultaneously present in these DAG-lactones was demonstrated earlier by the poor binding affinity shown by compounds lacking either one of these functionalities.[1]

B. Hydrophobic Interactions. Even though the role of the mostly linear aliphatic ester groups in DAG has been principally correlated with lipophilicity to facilitate partitioning or transport between biological phases,[34,35] the docking of the DAG-lactones to the C1b domain of PK-Cδ revealed potentially important hydrophobic contacts with the protein which could probably extend into the protein−membrane interface. Analysis of the *sn*-1 and *sn*-2 binding modes in relation to the location of a group of highly conserved hydrophobic amino acids on the rim of the two loops of the C1b domain in PKCδ (Met 239, Pro 241, Phe-243, Leu-250, Trp-252, and Leu 254)[27] provided the basis to modify the DAG-lactones in a fashion to optimize hydrophobic binding to these amino acids' side chains (vide infra). In the crystal structure of the phorbol-13-*O*-acetate/C1b complex of PK-Cδ, all these hydrophobic amino acids appear exposed and positioned to point into the membrane,[27] and in the case of the equivalent C1b domain of PK-Cγ, an NMR study of the amide proton signals perturbed by lipid interactions in the presence and absence phorbol-12,13-dibutyrate (PDBU) identified an equivalent set of hydrophobic amino acids on the top half of the C1b domain that is probably responsible for the insertion of the enzyme into the lipid membrane.[36]

Design and Synthesis

A. Design and Construction of the Branched Alkyl Chain. An inspection of the environment of the DAG-lactones docked in the C1b domain of PK-Cδ suggested that hydrophobic contacts with the protein might improve with the use of branched hydrocarbon

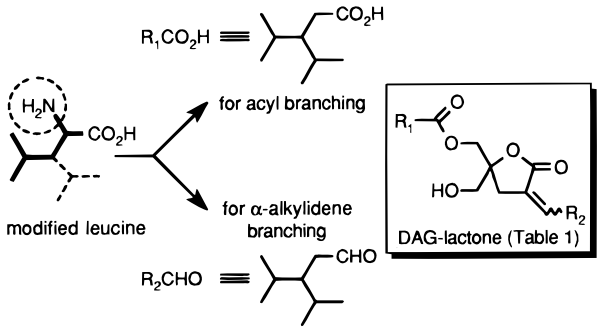
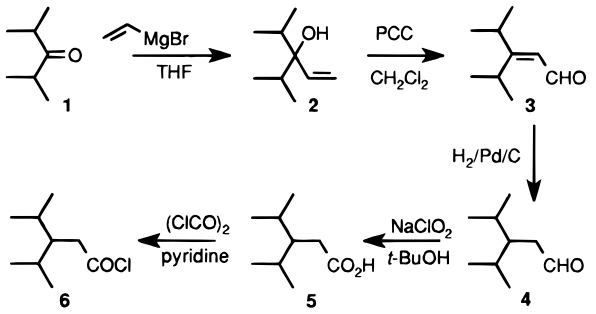


Figure 3. Design of the 2,3,4-trimethylpentane chain for acyl (R_1) and α-alkylidene (R_2) branching from the amino acid leucine. The operation involves the removal of the NH_2 group and the addition of an extra *i*-Pr group.

Scheme 1



chains instead of linear chains. For this purpose, a branched chain containing isopropyl groups was chosen to mimic the side chains of amino acids Leu 250, Leu 254, and Val 255 that are an integral part of the hydrophobic rim of the C1b domain.[27] The newly designed branched motif was conceived to be attached to the carbonyl group for acyl branching (R_1) or connected to the lactone via a methylene group for α-alkylidene branching (R_2) (Table 1). In designing an appropriate pattern, consideration was given to maintaining a symmetric branch capable of reaching hydrophobic binding sites in every orientation. For that reason, and also to increase hydrophobicity, the 2,3,4-trimethylpentane chain was selected. The process of selection and construction of this chain could be envisaged as derived from the amino acid leucine following the addition of a symmetrical isopropyl branch and the removal of the amino group as depicted in Figure 3.

The branched 2,3,4-trimethylpentane chain was constructed from diisopropyl ketone as shown in Scheme 1. Aldehyde **3** was prepared as reported by Denmark et al.,[37] and catalytic hydrogenation of **3** afforded the target aldehyde **4**. Because of their close structural similarity, both **3** and **4** were used as branched chains (R_2) for the α-alkylidene lactones (Table 1). Oxidation of the aldehyde provided the branched acid **5** which served as precursor of **6** for the acyl branched (R_1) lactones (Table 1).

A nice complementarity between hydrophobic groups in the branched DAG-lactone ligand and the C1b domain of PK-Cδ can be appreciated in Figure 4 for the doubly branched DAG-lactone **31** (Table 1). In the *sn*-1 binding mode (Figure 4, left), the α-alkylidene branched (R_2) motif appears to optimize hydrophobic contacts with the region between Met 239 and Leu 254, whereas the acyl branch interacts with the hydrophobic region defined by Leu 250 and Trp 252. In the alternative *sn*-2 binding mode, these interactions are reversed (Figure 4, right). The optimization of hydrophobic binding to the loops of the C1b domain derived from the branched chains of the DAG-lactones was expected to increase the binding affinity of the ligands in either alternative binding mode. However, since linear alkyl chains might interact better with the membrane, a structure−activity study with combinations of branched and linear chains was initiated to select the best blend of R_1 and R_2 substituents.

B. Design and Construction of Branched DAG-Lactones. 1. Racemic α-Alkylidene *E*- and *Z*-Lactones. The initial set of target compounds (**7**−**32**) is listed in Table 1. The first subgroup (compounds **7**−**12**) included compounds where aldehyde **3** was used for the construction of the α-alkylidene branch (R_2). To reach optimal lipophilicity and to ensure adequate partitioning into the membrane, a limited range (n = 1, 3, and 5) of linear aliphatic acyl groups ($R_1 = C_nH_{2n+1}$) was investigated. The second subgroup of compounds (**13**−**22**) included targets in which aldehyde **4** served as the precursor for the α-alkylidene branch. Since the *Z*-isomers **13**, **15**, and **17** showed increased potency vis-à-vis the *Z*-isomers of the first subgroup (vide infra), longer aliphatic acyl chains ($R_1 = C_nH_{2n+1}$, n = 7 and 9) were investigated in this series to optimize lipophilicity (compounds **19**−**22**). The third subgroup was designed to explore the effects of switching the branching from the α-alkylidene side to the acyl side. Therefore, each *Z*/*E*-pair in the third subgroup is isomeric and of equal lipophilicity to the corresponding *Z*/*E*-pair of the second subgroup. Finally, compounds **31** and **32** constitute the subgroup corresponding to doubly branched acyl and α-alkylidene lactones.

The compounds were synthesized as racemates using general alkylation and acylation procedures illustrated in Scheme 2. The corresponding geometrical *Z*- and *E*-isomers were separated, and the geometry of the exocyclic double bond was assigned by ^{1}H NMR spectroscopy. For the *E*-isomers **7**, **9**, and **11** (Table 1), the doublet corresponding to the γ-vinyl proton resonated at ca. δ 5.86, whereas the same signal for the *Z*-isomers (**8**, **10**, and **12**, Table 1) appeared 1.4 ppm lower at ca. δ 7.27. The *β-cis* vinyl proton of the *E*-isomers in the doubly unsaturated compounds (**7**−**12**), as well as in all the monounsaturated compounds (**13**-**32**, Table 1), appeared consistently ca. 0.5 ppm downfield from the corresponding *β-trans* proton of the *Z*-isomers.

2. Racemic α-Alkyl Lactones. Two additional compounds (**65** and **66**) corresponding to the *cis* and *trans* isomers that would have resulted from the catalytic hydrogenation of compounds **17**/**18** were synthesized (Scheme 3). These compounds were investigated to compare the effects of a branched α-alkyl moiety versus an α-alkylidene chain. Since these compounds represent a separate and limited structure−activity analysis, they are not included in Table 1. The precursor compound (**64**) could have been obtained by the catalytic hydrogenation of several intermediates (i.e., **52**−**55**); however, **53** turned out to be the most convenient starting material. Monoacylation of **64** gave a pair of diastereoisomers (**65** and **66**) in which the relative orientation of the acyl and α-alkyl branches could be either *cis* or

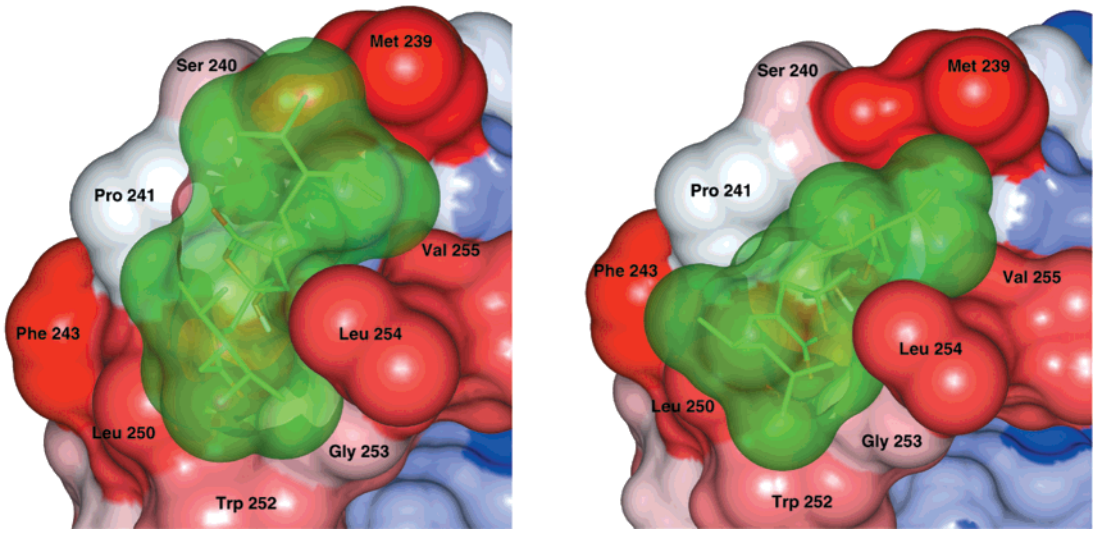
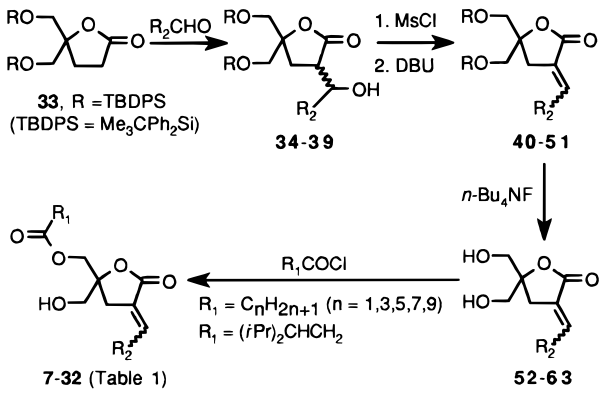


Figure 4. *sn*-1 (left) and *sn*-2 (right) binding modes for DAG-lactone **31** (Table 1) ($R_1 = R_2 = CH_2CH(i\text{-}Pr)_2$). The 3D surface representation was calculated with the program Insight 97, Molecular Simulation Inc. (http://www.msi.com/). The amino acid residues are colored according to the Insight 97 hydrophobicity scale (red, lipophile; blue, hydrophile).

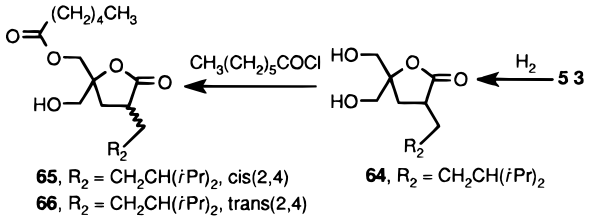
Scheme 2



#	R	R_2	Z/E	#	R	R_2	Z/E
34	TBDMS	CH=C(*i*-Pr)$_2$	—	49	TBDMS	$(CH_2)_4CH_3$	*E*
35	"	$CH_2CH(i\text{-}Pr)_2$	—	50	"	$(CH_2)_8CH_3$	*Z*
36	"	CH_3	—	51	"	$(CH_2)_8CH_3$	*E*
37	"	$(CH_2)_2CH_3$	—	52	—	CH=C(*i*-Pr)$_2$	*Z*
38	"	$(CH_2)_4CH_3$	—	53	—	CH=C(*i*-Pr)$_2$	*E*
39	"	$(CH_2)_8CH_3$	—	54	—	$CH_2CH(i\text{-}Pr)_2$	*Z*
40	"	CH=C(*i*-Pr)$_2$	*Z*	55	—	$CH_2CH(i\text{-}Pr)_2$	*E*
41	"	CH=C(*i*-Pr)$_2$	*E*	56	—	CH_3	*Z*
42	"	$CH_2CH(i\text{-}Pr)_2$	*Z*	57	—	CH_3	*E*
43	"	$CH_2CH(i\text{-}Pr)_2$	*E*	58	—	$(CH_2)_2CH_3$	*Z*
44	"	CH_3	*Z*	59	—	$(CH_2)_2CH_3$	*E*
45	"	CH_3	*E*	60	—	$(CH_2)_4CH_3$	*Z*
46	"	$(CH_2)_2CH_3$	*Z*	61	—	$(CH_2)_4CH_3$	*E*
47	"	$(CH_2)_2CH_3$	*E*	62	—	$(CH_2)_8CH_3$	*Z*
48	"	$(CH_2)_4CH_3$	*Z*	63	—	$(CH_2)_8CH_3$	*E*

trans. The orientation of the α-alkyl branch was ascertained by ^{1}H NMR spectroscopy which was sensitive to the relative anisotropy of the environment of the AB quartets corresponding to the acylated hydroxymethyl group [$CH_3(CH_2)COCH_2$] and the free CH_2OH group. The corresponding acylated hydroxymethyl group resonated consistently ca. 1 ppm lower than the free CH_2-OH group. Therefore, when the branched α-alkyl chain was located on the same side of either AB system, the spread of the AB quartet increased due to the restricted freedom of rotation. This simple observation was very diagnostic and provided an easy way to differentiate diastereoisomers **65** and **66**.

Scheme 3

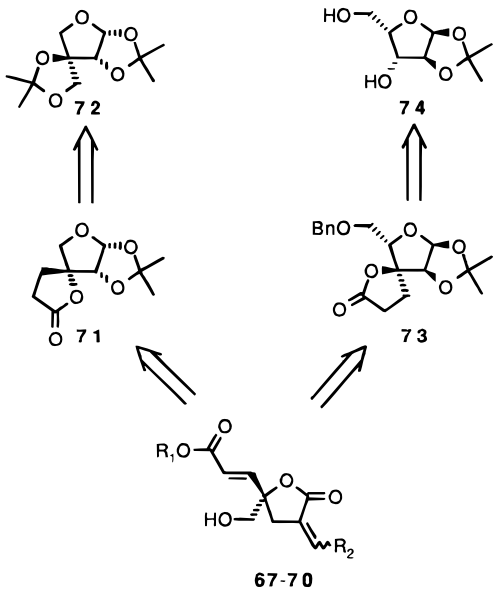


3. Enantiomeric α-Alkylidene *E*- and *Z*-Reverse Ester Lactones. We have previously shown that PK-C only recognizes DAG-lactones having the *R*-configuration.[25] However, since these compounds are susceptible to racemization via acyl migration, a series of "reversed ester" analogues were developed to overcome this problem.[26] This operation facilitated the synthesis of single enantiomeric targets which showed equivalent biological potencies to the corresponding "normal ester" enantiomers.[25,26] The most potent ligands developed earlier in this category were compounds **67** and **68**, which have an 18-carbon α-octadec-9-enylidene side chain selected to mimic oleic acid.[26] Compounds **67** and **68** (Table 2) are examples of this class of "reversed ester" analogues in which the relative position of the carbonyl ester remains unchanged. For these compounds, however, even though the relative stereochemistry between the "normal ester" and the "reversed ester" analogues is the same, the presence of the double bond in the latter group causes the chiral center to change from *R* to *S*.[26] As with lactones **13**−**22** (Table 1), the α-alkylidene branched 2,3,4-trimethylpentane (R_2) motif required lengthening the chain of the aliphatic ester (R_1) to improve overall hydrophobicity. Therefore, targets **69** and **70** (log P = 5.41, Table 2) were constructed with a six-carbon alkyl

Table 2. PK-Cα Binding Affinities (K_i) and log *P* for α-Alkylidene "Reversed Ester" Lactones (*Z*- and *E*-Isomers)

Compound	Cpd. #	*Z*-isomer K_i (binding) nM	Cpd. #	*E*-isomer Ki (binding) nM	log P octanol/water
R_1 = CH_3O; R_2 = $CH_3(CH_2)_7CH{=}CH(CH_2)_7$ (Z)	**67**	11 ±0.7	**68**	20 ±2.9	7.38
R_1 = $CH_3(CH_2)_5O$; R_2 = $CH_2CH(i\text{-}Pr)_2$	**69**	3.5 ±0.1	**70**	29.5 ±2.5	5.41

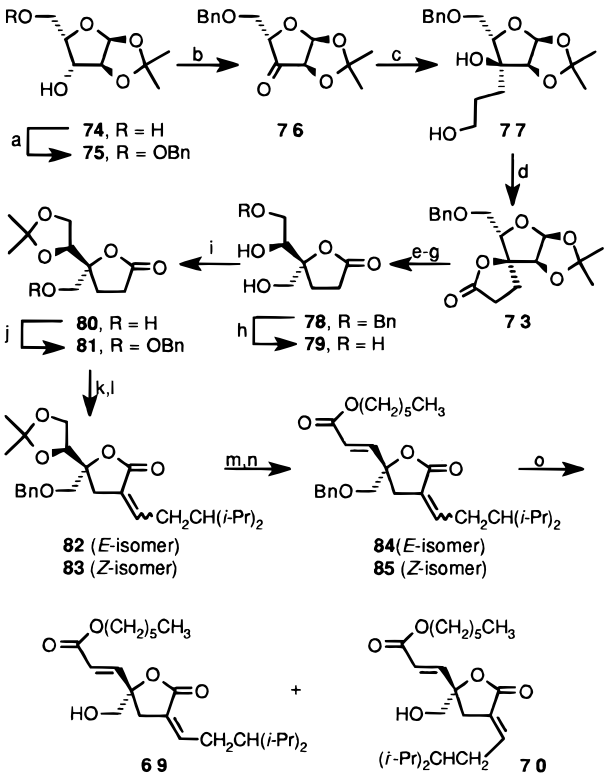
Scheme 4



chain designed to approximate the hydrophilic/lipophilic balance of compounds **19** and **20** (log P = 5.62, Table 1).

The already reported enantioselective syntheses of **67** and **68** (Table 2) started from spirolactone **71** constructed from the relatively expensive 1,2:3,5-di-*O*-isopropylidene-α-D-*threo*-apiofuranose (**72**).[26] Synthesis of an equally useful spirolactone synthon (**73**), obtained from cheaper L-xylofuranose (**74**), was developed for this project as a better alternative (Scheme 4). Hence, protected 1,2-*O*-isopropylidene-α-L-xylofuranose (**75**) was converted into spirolactone **73** as reported in a brief communication[38] and described here with full experimental details (Scheme 5). Monoprotection of the primary alcohol group in **74** as a benzyl ether (**75**) and oxidation to the 3-ulose intermediate (**76**) was followed by the stereoselective addition of the Grignard reagent ($ClMgO(CH_2)_3MgCl$) to the carbonyl from the less congested convex face of the molecule as directed by the 1,2-*O*-isopropylidene function. Lactone formation from **77** occurred in two steps via an intermediate lactol formed as the first oxidation product of the primary alcohol moiety with pyridinium chlorochromate (PCC). Further oxidation gave the key intermediate spirolactone **73**. The isopropylidene group was then hydrolyzed, and the resulting glycol moiety was cleaved with sodium metaperiodate to the corresponding aldehyde. Without isolation, the aldehyde was reduced with sodium borohydride to the alcohol with the concomitant hydrolysis of the formate ester. Hydrogenolysis of the benzyl group

Scheme 5[a]



[a] Reagents and conditions: (a) $(Bu_3Sn)_2O$, BnBr, Bu_4NBr, toluene (99%); (b) PDC, AcOH, 4 Å mol. sieve, CH_2Cl_2 (98%); (c) $Cl(CH_2)_3OH$, CH_3MgCl, Mg, $Br(CH_2)_2Br$ (85%); (d) PCC, 4 Å mol. sieve (98%); (e) Dowex H^+ resin, THF−H_2O; (f) $NaIO_4$, MeOH−H_2O; (g) $NaBH_4$, MeOH (75% in 3 steps); (h) H_2, Pd/C, MeOH (99%); (i) *p*-TsOH, acetone (97%); (j) BnBr, AgO, DMF (84%); (k) LiHMDS, THF, −78 °C; (*i*-Pr)$_2$CHCH$_2$CHO, HMPA; (l) MsCl, NEt_3, CH_2Cl_2, DBU (*Z*: 35%, *E*: 24%); (m) H_5IO_6, ether (96%); (n) $PPh_3{=}CHCO_2C_6H_{13}$, CH_2Cl_2 (93%); (o) BCl_3, CH_2Cl_2, −78 °C (50%).

gave the intermediate (5*R*)-5-[(1*S*)-1,2-dihydroxyethyl]-5-(hydroxymethyl)oxolan-2-one (**79**), which provided a chiral platform for the synthesis of the target compounds. Protecting the glycol moiety as the acetonide and the remaining primary alcohol as a benzyl ether allowed for complete differentiation between the two 5-substituents for later manipulation. Using the same general alkylation procedures illustrated in Scheme 2, the corresponding geometrical *E*-and *Z*-isomers (**82** and **83**) were obtained and separated chromatographically. The geometry of the exocyclic double bond was assigned in the same manner as before. Direct treatment of each individual isomer with periodic acid cleaved the acetonide group and oxidized the resulting glycol moiety to the aldehyde. The intermediate aldehyde was reacted immediately with hexyl (triphenylphosphoranylidene)-acetate to give the expected olefins **84** and **85**. Removal of the benzyl ether with BCl_3 afforded the two target geometrical isomers **69** and **70**.

Lipophilicity and Partition Coefficients

The octanol/water partition coefficients (log *P*) were calculated according to the fragment-based program KOWWIN 1.63[39] and are listed in Tables 1 and 2. This parameter was correlated with PK-C binding affinity (vide infra) by plotting log(1/K_i) versus log *P*. The four curves shown in Figure 5 represent the best quadratic

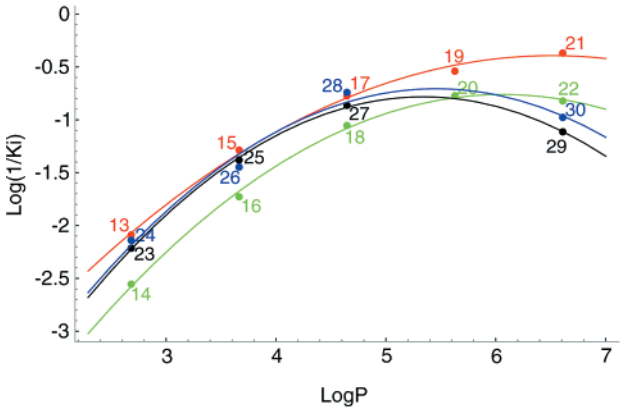


Figure 5. Binding affinity (log $1/K_i$) versus partition coefficients (log P) in octanol/water. The red (Z-isomers) and green (E-isomers) curves correspond to the α-alkylidene branched series (**13**−**22**), and the black (Z-isomers) and blue (E-isomers) curves correspond to the acyl branched series (**23**−**30**).

fit calculated for two subgroups (Z- and E-isomers) of DAG-lactones selected from Table 1. For the α-alkylidene branched series (**13**−**22**), the curves revealed that affinity for PK-C reaches a maximum at ca. log $P = 6$ for both Z- and E-isomers. In these curves, a consistent greater affinity for the Z-isomers can be appreciated. For the acyl branched series (**23**−**30**), a similar plot revealed that PK-C affinity starts to drop much earlier at ca. log $P = 5$. Moreover, the difference between geometrical isomers appeared smaller and was reversed relative to the α-alkylidene branched series.

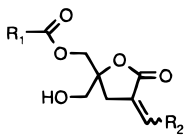
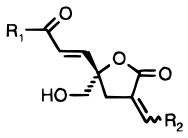
The doubly branched compounds (**31** and **32**) have log P values 0.72 lower than the most lipophilic compounds in the two previous subgroups and showed no apparent difference between geometric isomers in terms of PK-C binding (Table 1). These compounds appear to have the most favorable log($1/K_i$)/log P ratio compared to the most potent α-alkylidene analogues (**21** and **22**). From these data it can be concluded that an optimal log P for these DAG-lactones lies between 5 and 6.

The "reversed ester" lactones featured in Table 2 showed that the α-alkylidene branched Z-isomer (**69**) was the most potent ligand with the lowest log P value (5.41). It is significant that the set of isomers **69**/**70** is almost 2 log P units less lipophilic than the **67**/**68** pair (Table 2), and yet the Z-isomer **69** is 3 times more potent than Z-isomer **67**. For comparison, phorbol 12,13-dibutyrate (PDBU, $K_i = 0.2$ nM)[40] and prostratin (12-deoxyphorbol-13-acetate, $K_i = 4.8$ nM)[40] have calculated log P values of 3.43 and 3.50, respectively.

Molecular Modeling

DAG-lactones **9** and **10** [α-alkylidene branched, R_2 = CH=C(i-Pr)$_2$], **15** and **16** [α-alkylidene branched, R_2 = $CH_2CH(i\text{-Pr})_2$], **25** and **26** [acyl branched, R_1 = $CH_2CH(i\text{-Pr})_2$], **31** and **32** [doubly branched, $R_1 = R_2$ = $CH_2CH(i\text{-Pr})_2$], and **69** and **70** ["reversed esters", R_1 = $CH_3(CH_2)_5O$, R_2 = $CH_2CH(i\text{-Pr})_2$] were docked into the empty PK-Cδ C1b domain using the program Autodock 2.4[41] (Table 3). This program combines a Monte Carlo simulated annealing algorithm to search the conformational space with a fast evaluation of the interaction energy. The search strategy employed by the program is a random walk of the ligand on the surface of the receptor, which is kept rigid. During the search, the

Table 3. Percent of Docking Simulations in *sn*-1 and *sn*-2 Binding Modes

	Cpd. #	K_i nM	Isomer	*sn*-1 mode	*sn*-2 mode	other
R_1 = $CH_3(CH_2)_2$ R_2 = CH=C(i-Pr)$_2$	**9**	68 ±6.0	*Z*	12	87	1
R_1 = $CH_3(CH_2)_2$ R_2 = CH=C(i-Pr)$_2$	**10**	51 ±8.0	*E*	63	37	0
R_1 = $CH_3(CH_2)_2$ R_2 = $CH_2CH(i\text{-Pr})_2$	**15**	19 ±1.5	*Z*	44	56	0
R_1 = $CH_3(CH_2)_2$ R_2 = $CH_2CH(i\text{-Pr})_2$	**16**	54 ±4.0	*E*	7	75	18
R_1 = $CH_2CH(i\text{-Pr})_2$ R_2 = $CH_3(CH_2)_2$	**25**	24 ±1.0	*Z*	41	58	1
R_1 = $CH_2CH(i\text{-Pr})_2$ R_2 = $CH_3(CH_2)_2$	**26**	28 ±6	*E*	44	52	4
$R_1 = R_2$ = $CH_2CH(i\text{-Pr})_2$	**31**	2.9 ±0.2	*Z*	63	32	5
$R_1 = R_2$ = $CH_2CH(i\text{-Pr})_2$	**32**	2.7 ±0.4	*E*	22	57	21
	Cpd #	K_i nM	Isomer	*sn*-1 mode	*sn*-2 mode	other
R_1 = $CH_3(CH_2)_5O$ R_2 = $CH_2CH(i\text{-Pr})_2$	**69**	3.5 ±0.1	*Z*	0	87	13
R_1 = $CH_3(CH_2)_5O$ R_2 = $CH_2CH(i\text{-Pr})_2$	**70**	29.5 ± 2.5	*E*	0	87	13

temperature is gradually reduced, while the ligand translates, rotates, and changes its conformation. AutoDock provides a simple molecular mechanics force field to calculate the intra- and intermolecular energies. The ligands do not have hard degrees of freedom, i.e., bond stretching and angle bending are not allowed, and rings are kept rigid. By default, the force field does not provide 1,4-torsional parameters and relies upon nonbonded interactions only. van der Waals interactions are calculated using a Lennard-Jones 12-6 potential, whereas the hydrogen-bonding term is modeled by Lennard-Jones directional 12-10 potential. Electrostatics are calculated with a distance-dependent dielectric function. To speed the calculations of intermolecular energies between the macromolecule and the ligands, nonbonded interactions in the space surrounding the active site are precalculated and stored in three-dimensional grids, one for each atom type of the ligand. Docking simulations were performed 100 times with different random seeds to ensure the convergence of the results. Available within the program is a clustering utility that was used to group together similar docked conformations.

Using this procedure, the crystallographic position of phorbol-13-*O*-acetate in the C1b domain was duplicated 49 times out of 100 docking runs with a root-mean-square-deviation (RMSD) ranging from 0.59 to 0.76 Å. The lowest energy docking with a RMSD of 0.72 Å

reproduced well the position, orientation, and hydrogen-bonding network of the original crystal structure.[27] Except for the "reversed ester" lactones **69** and **70**, which docked almost exclusively in an *sn*-2 mode, the program engendered both *sn*-1 and *sn*-2 binding modes in different ratios depending on the size, stereochemistry, and shape of the R_1 and R_2 groups on the DAG-lactones (Table 3). Both *sn*-1 and *sn*-2 binding modes had a comparable AutoDock scoring energy and displayed a similar network of hydrogen bonds to Thr 242, Leu 251, and Gly 253, as observed with phorbol-13-*O*-acetate,[27] with participation of the primary OH and one of the carbonyl functions (Figure 2, yellow dotted lines). The combined total for the *sn*-1 and *sn*-2 binding modes ranged from 79% to 100% for the series of 100 docking runs, with the remaining docking runs corresponding to energetically less favorable complexes.

There are no exceptionally revealing trends in Table 3 that could be strictly correlated with biological activity (vide infra). However, in the case of the α-alkylidene branched lactones with a linear acyl chain (**9**, **10**, **15**, and **16**), the preference for a specific binding mode appears to be more sensitive to the differences in stereochemistry about the α-alkylidene double bond. On the other hand, the difference in preference between *Z*- and *E*-isomers for acyl branched lactones **25** and **26** becomes minimal. This observation agrees with the data plotted in Figure 5, where differences between geometrical isomers were consistently more pronounced for the α-alkylidene branched series. One of the most important conclusions from these docking experiments, however, is that both *sn*-1 and *sn*-2 binding modes were observed, except in the case of the "reversed ester" lactones **69** and **70**, where the *sn*-2 binding mode is preferred.

Biological Results and Discussion

A. PK-C Binding Affinity. The interaction of the target DAG-lactones (**7**−**32** and **65**−**70**) with PK-C was assessed in terms of the ability of the ligand to displace bound [20-^{3}H]phorbol 12,13-dibutyrate (PDBU) from a recombinant single isozyme (PK-Cα) in the presence of phosphatidylserine as already described.[24−26,42] The inhibition curves obtained for all ligands were of the type expected for competitive inhibition, and the ID_{50} values were determined by fit of the data points to the theoretical noncooperative competition curve. The K_i's for inhibition of binding (Tables 1 and 2) were calculated from the ID_{50} values. The K_i values for the first subgroup of DAG-lactones (**7**−**12**, Table 1) which have a 4-methyl-3-(methylethyl)pent-2-enylidene [CH═C(*i*-Pr)$_2$] branched chain at R_2 showed that there is a critical need for an optimal overall hydrophobicity to produce a stable complex with the enzyme. Indeed, for a constant and invariant set of polar groups on the lactone moiety that are responsible for receptor recognition and hydrogen bonding, the expected interaction of the branched chain with the hydrophobic regions of the C1 domain became evident only after reaching an adequate level of lipophilicity (log P = 4.56) with a five-carbon linear acyl chain [$R_1 = (CH_2)_4CH_3$]. This level of lipophilicity was probably necessary for effective partitioning into the phospholipid phase. Remarkably, the two most potent ligands of this subgroup (compounds **11** and **12**) are equal or better than the heretofore most potent DAG-lactones **67** and **68** (Table 2), but with six fewer carbon atoms and 2.82 log P units lower. This suggests that binding affinity in this series probably increased by improving hydrophobic interactions with the C1 domain and not just by a coarse increase in lipophilicity. As observed with other DAG-lactones, PK-C binding affinity for this series was also sensitive to the steric disposition of the α-alkylidene chain, but contrary to the more typical behavior,[24−26] it was the *E*-isomers that showed a slight advantage.

The DAG-lactones belonging to the second subgroup in Table 1 (compounds **13**−**18**), which have a branched 4-methyl-3-(methylethyl)pentylidene [$(CH_2CH(i\text{-}Pr)_2$] chain at R_2, showed similar or improved affinities vis-à-vis the equivalent compounds of the first subgroup (**7**−**12**). Also, these compounds showed the more characteristic behavior where the *Z*-isomers possessed higher affinities (ca. > 2-fold) than the corresponding *E*-isomers.[22−24] Longer acyl chains ($R_1 = C_nH_{2n+1}$, n = 7 and 9) were investigated to optimize partitioning into the phospholipid phase (compounds **19**−**22**). However, despite the improved affinities achieved as the length of R_1 increased, the effect was relatively modest and not commensurate with the number of carbons (Figure 5).

Swapping the position of the branched chain from R_2 to R_1 (compounds **23**−**30**, third subgroup, Table 1) was not as effective. Although some potent compounds were discovered, it appears that branching at R_1 does not have the same favorable outcome as branching at R_2. The earlier decline in affinity observed for a similar range of log P values (Figure 5) suggests that the acyl branch chain at R_1 is probably binding at a different site compared to the same type of branch at R_2. In addition, differences between geometrical isomers were less pronounced and showed instead a slight preference (ca. 1.2) for the *E*-isomers.

Having both R_1 and R_2 with equally branched chains gave two of the most potent compounds (**31** and **32**, Table 1), comparable or slightly better than compounds **19**−**22** of the second subgroup, but 0.72 log P units less lipophilic. Compounds **31** and **32** have similar lipophilic groups at both R_1 and R_2 and showed virtually no difference between geometrical isomers in terms of binding affinity.

Conversion of the α-alkylidene branch into an α-alkyl branch was briefly explored. Diastereoisomers **65** and **66** (Scheme 3) showed that a branched α-alkyl moiety was not as effective as an α-alkylidene chain. The K_i values (nM) for these compounds were, respectively, 20 ± 1.7 (*cis*-isomer **65**) and 23 ± 0.8 (*trans*-isomer **66**), significantly poorer than **17** (5.9 ± 0.2) and **18** (11 ± 0.6).

Finally, the two homochiral DAG-lactones **69** and **70** (log P = 5.41, Table 2), which were designed to have a hydrophobic/hydrophilic balance similar to that of the potent DAG-lactone racemates **19** and **20** (log P = 5.62, Table 1), showed the largest difference (8.5-fold) so far observed between two geometrical isomers. In agreement with the modeling data (Table 3), this difference suggests that there is a more specific and discriminating interaction between each isomer and the C1 domain/lipid complex of PK-C. The close agreement between the measured K_i values for *Z*-isomers **69** and **19** is in

keeping with our previous report on the bioequivalence of "reversed ester" DAG-lactones and conventional DAG-lactones.[26] Assuming that both compounds bind at a common site, one would have expected the active enantiomer **69** to be twice as potent as the racemate **19**. However, in reality these compounds are structurally distinct and, as discussed in the modeling section, they probably bind differently (i.e., mixed *sn*-1/*sn*-2 mode for **19** versus pure *sn*-2 mode for **69**).

A structure−activity analysis that correlated PK-C binding affinity to a specific binding preference, *sn*-1 or *sn*-2, could not be formulated. As shown in Figure 3, when the doubly branched DAG-lactone **31** is docked into the empty C1b domain of PK-Cδ, both branched lipophilic ends of the molecule in either binding mode can cover effectively the rim of the C1b domain. It is therefore possible that after van der Waals interactions between the segments of the chain involved in hydrophobic binding to the protein, the remainder of the chain, linear or branched, could protrude outside into the membrane. Since it is logical to assume that interactions with the protein would be more sterically sensitive than interactions with the membrane, the more rigid α-alkylidene chain would show greater differences in binding between *Z*- or *E*-isomer when directly involved with the protein. The groove between the loops formed by Met 239 and Leu 254 in the C1b domain is more sterically constrained than the alternative site near Leu 250 and Trp 252. Therefore, the specific preference for a particular binding mode will determine which end of the molecule is to occupy the more constrained site. For DAG-lactones **13**−**22** (Table 1), the *sn*-1 binding mode directs the α-alkylidene branch into the more sterically demanding groove between Met 239 and Leu 254. Hence, in this binding mode, the differences in K_i values between *Z*- and *E*-isomers would be expected to be larger as was experimentally observed (Figure 5). On the other hand, the results for the acyl branched DAG-lactones (**23**−**30**, Table 1) could be explained by both binding alternatives. In an *sn*-1 binding mode, the linear α-alkylidene (R_2) branch would lie inside the Met 239 and Leu 254 groove, but the lack of branching would result in a less efficient discrimination between geometric isomers as experimentally observed (Figure 5). In the alternative *sn*-2 binding mode, it would be the branched acyl (R_1) chain occupying the Met 239 and Leu 254 groove, but since there are no geometrical restrictions, the chain is free to accommodate into an optimal disposition. At the other end, the linear α-alkylidene (R_2) chain would reside in the less sterically demanding shallow groove near Leu 250 and Trp 252 where little differences between geometrical isomers would be expected.

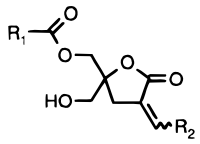
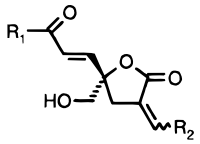
According to the docking results and the above arguments, both competing binding modes are reasonable, and the final selection is probably controlled by the lipophilic imbalance between the two chains and how they interact with the lipid phase.

The "reversed ester" DAG-lactones **67**−**70** (Table 2) are an interesting case in that they are more conformationally restricted. In addition to the α-alkylidene chain (R_2), the "reversed ester" moiety also forms part of an unsaturated chain. It is important to recall that, according to our previous work, this additional unsaturation was necessary to match the binding affinity to that of the corresponding conventional DAG-lactones.[26] Docking experiments with AutoDock showed that these "reversed ester" DAG-lactones bind preferentially in the *sn*-2 binding mode. Remarkably, for compounds **69** (*Z*-isomer) and **70** (*E*-isomer), the difference in binding affinity increased by more than 8-fold, relative to the ca. 2-fold difference observed for the rest of the DAG-lactones.

B. PK-C Binding Affinity versus Lipophilicity. The most potent DAG-lactones in either branched α-alkylidene or branched acyl series appear to have an adequate lipophilic/hydrophilic balance to effectively localize into lipid bilayers. It is possible that in such an environment these amphiphilic molecules, regardless of their structure, may engage in some nonspecific interaction with PK-C. However, when the hydrophilic groups on the lactone template are additionally capable of fitting well into the C1 domain of the enzyme, a significant increase in binding affinity is observed. What should therefore be the optimal relationship between lipophilicity and potency? Comparing the most potent DAG-lactones from this study (**21**, **31**, **32**, and **69**) with some prototypic phorbol esters, we find that the DAG-lactone ligands are ca. 10-fold less potent than PDBU (K_i = 0.2−0.8 nM) and ca. 2-fold more potent than prostratin (12-deoxyphorbol-13-acetate, K_i = 4.8 nM), a non-tumor-promoting phorbol ester. The calculated log *P* values for PDBU and prostratin are very close (3.43 and 3.50, respectively) and are almost two log *P* units lower than the least lipophilic lactone (compound **69**, log *P* = 5.41). This means that relative to the natural phorbol esters, it is still possible to modify the structures of the DAG-lactones with different and smaller branched motifs that could reduce lipophilicity and maintain or increase binding affinity.

C. PK-C Activation. Direct stimulation of phosphorylation of the standard α-pseudosubstrate peptide by PK-Cα was measured for a selected group of compounds (Table 4). When interpreting the results, it is important to realize that the sensitivity of this assay is significantly less than that of the binding assay, being carried out under conditions of limiting phospholipid. Despite this caveat, some salient points deserved to be mentioned. For this assay, three *Z* -isomers (**15**, **17**, **21**) from the α-alkylidene branched group, two *Z*-isomers (**27** and **29**) from the acyl branched group, and the two doubly branched *Z*- and *E*-isomers (**31** and **32**) from Table 1 were selected, together with *Z*-isomers **67** and **69** from the "reversed ester" group in Table 2. One striking observation for the α-alkylidene branched group is that the ED_{50}'s for activation do not strictly correlate with PK-C binding affinities which are determined under conditions of saturating anionic phospholipid. The ED_{50} values for the α-alkylidene branched group, which have a linear acyl chain (R_1), are the weakest enzyme activators with an ED_{50} range of 0.6 to 2.3 μM. Moreover, only small differences were observed when the linear acyl chain R_1 was increased from three to nine carbons, although in the end the most lipophilic compound (**21**) was the most potent of the three. With acyl branching, on the other hand, enzyme activation improved and the ED_{50} values were reduced to less than 0.5 μM compared to the set of equally lipophilic com-

Table 4. PK-Cα Activation by Selected DAG-Lactones

	Cpd. #	K_i nM	PK-C activation ED_{50} μM	EGF inhibition ED_{50} μM
$R_1 = CH_3(CH_2)_2$ $R_2 = CH_2CH(i\text{-}Pr)_2$	**15**	19 ±1.5	1.95 ±0.16	0.82 ±0.12
$R_1 = CH_3(CH_2)_4$ $R_2 = CH_2CH(i\text{-}Pr)_2$	**17**	5.9 ±0.2	2.33 ±0.14	2.22 ±0.65
$R_1 = CH_3(CH_2)_8$ $R_2 = CH_2CH(i\text{-}Pr)_2$	**21**	2.3 ±0.1	0.66 ±0.11	1.17 ±0.09
$R_1 = CH_2CH(i\text{-}Pr)_2$ $R_2 = CH_3(CH_2)_4$	**27**	7.3 ±0.5	0.34 ±0.04	0.13 ±0.06
$R_1 = CH_2CH(i\text{-}Pr)_2$ $R_2 = CH_3(CH_2)_8$	**29**	13 ±0.4	0.43 ±0.02	0.37 ±0.04
$R_1 = R_2 = CH_2CH(i\text{-}Pr)_2$	**31**	2.9 ±0.2	0.55 ±0.13	0.17 ±0.05
$R_1 = R_2 = CH_2CH(i\text{-}Pr)_2$	**32**	2.7 ±0.4	0.68 ±0.27	0.14 ±0.06
	#	K_i nM	PK-C activation ED_{50} μM	EGF inhibition ED_{50} μM
$R_1 = CH_3O$ $R_2 = (CH_2)_7CH$ $\|\|$ $CH_3(CH_2)_7CH$	**67**	11 ±0.7	1.07 ±0.26	0.33 ±0.08
$R_1 = CH_3(CH_2)_5O$ $R_2 = CH_2CH(i\text{-}Pr)_2$	**69**	3.5 ±0.1	0.95 ±0.06	0.14 ±0.05
PDBU		0.2 ± 0.03	0.02 ±0.003	0.02 ±0.01

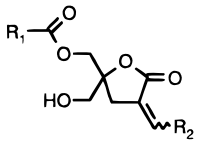
pounds in the α-alkylidene branched group (compare **17** with **27** and **21** with **29**). Within experimental error, the doubly branched *Z*- and *E*-isomers (**31** and **32**) can be considered equally potent to the acyl branched DAG-lactones **27** and **29**. Finally, enantiomers **67** and **69** from the "reversed ester" group had similar ED_{50} values close to 1 μM.

As PK-C activators, the most potent of the DAG-lactones investigated appears to be about 1 order of magnitude less potent than PDBU (ED_{50} = 0.02 μM). The lack of good correlation between binding affinity and PK-C activation within the DAG-lactones themselves [compare compounds **17** (K_i = 5.9 nM) and **27** (K_i = 7.3 nM) in Table 4] may be due to the more dominant role played by the physical properties of the membrane in the PK-C activation assay. Although membrane interactions were not part of the structure−activity analysis in the receptor-guided approach, the data seem to suggest that a branched acyl chain at R_1 is preferable for enzyme activation. Therefore, under the conditions of limiting lipid used in the PK-C activation assay, membrane interactions may override the weaker van der Waal interactions with the C1 domain of the enzyme that were optimized with α-alkylidene branching. In the case of the doubly branched analogues (**31** and **32**), since both R_1 and R_2 groups are of similar structure and lipophilicity, their mode of binding may alternate between the *sn*-1 and *sn*-2 modes since they are probably not effectively discriminated by the membrane. Also, it is possible that during PK-C activation the balance between the two binding modes might change when the enzyme−ligand complex moves from the aqueous phase to the lipid phase during translocation. Finally, in the case of the "reversed ester" lactones, which probably assume a more exclusive *sn*-2 binding mode, good activation was seen albeit somewhat lower than with compounds **27**, **29**, **31**, and **32**.

D. EGF Binding Inhibition. The epidermal growth factor (EGF) receptor binds EGF, causing dimerization and unleashing of tyrosine-specific kinase activity which leads to autophosphorylation and downstream responses coupled to autophosphorylation.[29] Among the well-characterized inhibitors of EGF binding are the phorbol esters,[43] which act indirectly on EGF binding through activation of PK-C. The ED_{50} values for inhibition of EGF binding by the DAG-lactones paralleled closely the results on PK-C activation (Table 4). As with PK-C activation, the simple α-alkylidene branched DAG-lactones with simple linear acyl chains were again the weakest inhibitors (compounds **15**, **17**, and **21**). Acyl branching, on the other hand, led to lower ED_{50} values. As seen with PK-C activation, an increase in the lipophilicity of the linear α-alkylidene chain was not accompanied by a proportional reduction in the ED_{50} value (compare compound **27** and **29**). The doubly branched compounds (**31** and **32**) were again equivalent and performed as well as the best acyl branched compound **27**. Finally, among the single enantiomers, compound **69** was as potent as **27** and the **31**/**32** pair. Since inhibition of EGF binding is an indirect effect of PK-C activation in whole cells, it is difficult to postulate a direct structure−activity correlation. However, as observed with PK-C activation, it appears that the nature of the acyl branch becomes more dominant in an assay that is additionally sensitive to issues such as uptake, transport, and stability of the acyl chains to the action of esterases.

E. Antitumor Activity. The majority of the DAG-lactones were tested in the NCI in vitro primary screen which consists of a panel of 60 different tumor cell lines.[30] The results corresponding to the three compounds that gave reproducible results in two separate screens (compounds **29**, **31**, and **32**) are presented in Table 5. These compounds have been selected by the NCI for referral to the Biological Evaluation Committee.[44] A compound rejected by the screen (compound **21**) with comparable lipophilicity to the other three was included for comparison. All the compounds in this table have similar lipophilicities with log *P* values of 5.89 (**31** and **32**) and 6.61 (**21** and **29**). The first data entry in Table 5 represents the mean 50% growth inhibition parameter (GI_{50}) for the entire panel of cells (MG-MID). The next column (the so-called Delta value)[45] shows the number of log units by which the most sensitive line of the panel differs from the corresponding mean for the entire cell panel. Inspection of the first two data columns shows that compound **31** displayed excellent specificity with a Delta value of 2.51. The following three columns

Table 5. Results of Comparative Testing in the NCI in Vitro Screen

	#	Full Panel MG-MID[a]	Delta[b]	Leukemia K-562 (GI_{50})[c]	Colon COLO 205 (GI_{50})[c]	Breast HS 578T (GI_{50})[c]	COMPARE[d] GI_{50} correlation coefficient to Prostratin
$R_1 = CH_3(CH_2)_8$ $(Z)\text{-}R_2 = CH_2CH(i\text{-}Pr)_2$	**21**	4.4×10^{-5}	0.68	1.8×10^{-5}	9.2×10^{-6}	2.7×10^{-5}	—
$R_1 = CH_2CH(i\text{-}Pr)_2$ $(Z)\text{-}R_2 = CH_3(CH_2)_8$	**29**	4.7×10^{-6}	1.91	7.1×10^{-8}	1.4×10^{-7}	5.8×10^{-8}	0.690
$R_1 = (Z)\text{-}R_2 = CH_2CH(i\text{-}Pr)_2$	**31**	3.2×10^{-6}	2.51	2.5×10^{-8}	2.1×10^{-8}	2.6×10^{-8}	0.720
$R_1 = (E)\text{-}R_2 = CH_2CH(i\text{-}Pr)_2$	**32**	1.0×10^{-5}	0.84	1.6×10^{-6}	1.5×10^{-6}	1.2×10^{-5}	—
Prostratin		7.0×10^{-6}	1.25	—[e]	1.9×10^{-6}	7.9×10^{-7}	1.000

[a] MG-MID is the calculated mean panel GI_{50} concentrations (M). [b] Delta is the number of $\log_{10}$ units by which the delta of the most sensitive line(s) of the panel differs from the corresponding MG-MID. The individual deltas are calculated by subtracting each $\log_{10}$ GI_{50} from the panel mean. [c] GI_{50} is an interpolated value representing the concentration (M) at which percentage growth is inhibited 50%. [d] COMPARE is a computerized, pattern-recognition algorithm to determine the degree of similarity (see text). [e] This cell line was tested in a different experiment ($GI_{50} = 5.0 \times 10^{-7}$ M). The GI_{50} profiles of the individual compounds were used as "seeds" against the historical NCI database.

include data for the three most sensitive cell lines from the leukemia, colon, and breast subpanels. On the basis of the results from the cellular assays in Table 4, it is not surprising that the α-alkylidene DAG-lactone **21** (log $P = 6.61$) with a linear acyl chain (R_1) failed to show antitumor activity despite having one of the lowest K_i values (2.3 nM, Table 1) in the PK-C binding assay. On the other hand, notwithstanding being a weaker PK-C ligand, DAG-lactone **29** (K_i = 13 nM, log $P = 6.61$) displayed important antitumor activity and selectivity resulting simply from the switching of the branched acyl and α-alkylidene groups. The doubly branched compound **31** was undoubtedly the best compound, demonstrating the advantage of both branched α-alkylidene and acyl ester groups. It is intriguing, however, that despite having similar K_i's in the binding assay (Table 1) and similar ED_{50}'s in PK-C activation and inhibition of EGF binding (Table 4), the change in the geometrical disposition of the α-alkylidene chain from the *Z*-(**31**) to the *E*-isomer (**32**) had such a dramatic effect in antitumor activity. This change in sensitivity, reflected in the value of Delta, serves as a reminder once more that membrane effects, which are not accounted for by any of the models employed to date, are very important.

Finally, the COMPARE program was utilized to probe the historical NCI database for compounds having an activity profile similar to the two best compounds **29** and **31**. COMPARE is a computerized, pattern-recognition algorithm that determines the degree of similarity, or lack thereof, of mean-graph profiles generated by the screen.[46] The response profile fingerprints of compounds **29** and **31** were used as "seeds" to find closely matching profiles contained in the NCI database. One of the most important uses of COMPARE is to identify compounds of unrelated chemical structure that share a related biochemical mechanism of action. The result is a rank-ordered list of similarity which is expressed in terms of a correlation coefficient. We were pleased to see that one of the highest ranked compounds (the highest for compound **31** and third highest for compound **29**) was prostratin (12-deoxyphorbol-13-acetate), a non-tumor-promoting antitumor agent.[40,47] *The antitumor results show that despite our limited understanding of how the membrane modulates PK-C activity, the equivalent spatial disposition of pharmacophores in the DAG-lactones and the phorbol nucleus, which has been the paradigm in our pharmacophore- and receptor-guided approaches, is corroborated by a completely unbiased analysis of the antitumor data in the NCI screen.* Of additional interest was that other phorbol-like structures were identified by COMPARE. For example, highly correlated to **31** were 13,20-diacetyl-12-octadienoyl-4-deoxyphorbol (correl. coeff. = 0.704), 20-acetyl-13-isobutyryl-12-(2,4-octadienoic acid)-4-deoxyphorbol (correl. coeff. = 0.662), merezein (correl. coeff. = 0.650), and an ingenol analogue (correl. coeff. = 0.627). Furthermore, a direct comparison between prostratin[48] and the best DAG-lactone **31** demonstrated that the latter is more potent and selective (Table 5). Because of PK-C's central role in signal transduction, the enzyme has been an attractive target for therapeutic intervention,[49] although efforts have been directed mostly to inhibiting the enzyme with catalytic-targeted inhibitors.[50] On the other hand, despite two reported clinical uses of phorbol esters,[51,52] their close association with tumor promotion has prevented their development as drugs. The already reported strong antipromoting effect of prostratin, coupled with its antitumor potential, has allayed fears that PK-C activators are necessarily tumor promoters.[53] Furthermore, bryostatin 1, which is a potent PK-C activator, is currently in clinical trials[14] and like prostratin fails to promote.[54] The results achieved here with simple DAG analogues, which are structurally unrelated to the phorbol esters, should provide a stronger incentive to explore the role of PK-C activators in the control of neoplasia.

Conclusions

1. The initial pharmacophore-guided approach that was based on the structure of the phorbol esters

culminated with the successful design of structurally simple and potent DAG surrogates (DAG-lactones). In this work, the pharmacophore-guided approach was refined to a higher level with information gathered from the X-ray structure of the C1b domain of PK-Cδ complexed with phorbol-13-*O*-acetate. The basis of this refinement, characterized as the receptor-guided approach, helped identify regions among a group of highly conserved hydrophobic amino acids along the rim of the C1b domain that allowed the implementation of important structural modifications on the simple DAG-lactone structure. In essence, the minimal "recognition domain" provided by the DAG-lactone was modified with a lipophilic "affinity domain" constituted mainly of branched α-alkylidene or acyl chains that drove binding affinities into the low nanomolar range. The branched alkyl chains appear to improve binding at two distinct levels. They appear to facilitate important van der Waals contacts with hydrophobic segments of the protein and help promote the activation of PK-C through critical membrane interactions. The latter may involve facilitating bilayer-to-hexagonal phase transitions resulting from an increase in headgroup spacing.

2. From the standpoint of a structure−activity analysis, the most important change in the progression from the pharmacophore-guided approach to the receptor-guided approach was that the latter suggests the existence of competing *sn*-1 and *sn*-2 binding modes. In the pharmacophore-guided approach, the best correlation obtained from the superposition of either DAG or DAG-lactones to phorbol esters favored the *sn*-1 binding modality. This is in direct contrast with the "reversed ester" DAG-lactones which appear to prefer the *sn*-2 mode. Further distinction between these two binding modes will require a combinatorial assembly with a constant "recognition domain" modified with a library of unsymmetrically branched hydrophobic "affinity domains".

3. The structural simplicity of the potent DAG-lactones should dispel the myth that chemical complexity and pharmacological potency go hand in hand. Even as racemates, the DAG-lactones show low namomolar binding affinities for PK-C and display selective antitumor activity at equivalent nanomolar levels. This is a welcome finding for future efforts to exploit molecular diversity for PK-C and other high-affinity receptors for DAG and the phorbol esters. DAG is now known to interact with four classes of high-affinity receptors, comprising a total of 15 family members identified so far.[55] The diverse family of PK-C isozymes, because of their ubiquitous distribution and extensive characterization, represent the primary challenge. The potential for generating massive numbers of simple DAG-lactone agonists compares favorably with the more complex task of simplifying the structures of natural products, such as the bryostatins[15−17] and teleocidins.[18−21] Finally, a completely unbiased approach using the COMPARE program to analyze the antitumor profile of the DAG-lactones has corroborated the use of our pharmacophore- and receptor-guided approaches. This effort demonstrates how this combined approach can produce a novel class of potentially useful antitumor DAG-lactones from a set of first principles.

Experimental Section

Molecular Modeling and Docking Experiments. An in-house modified version of the AutoDock 2.4 package was used. The source code was improved such that all variables are initialized, array boundary errors and memory corruption are avoided, and the input files are better validated. Memory usage is optimized by allocating memory dynamically for the grids. The implementation of the program Autotors, which is used to interactively select rotatable bonds in the ligand, was modified to automatically deselect bonds that involve a pair of conjugated atoms. The crystal coordinates of the C1b domain complexed with phorbol-13-acetate were retrieved from the Brookhaven Protein DataBank (code 1ptr). Using Quanta 97,[56] the phorbol-13-acetate was removed and polar hydrogens and template-based partial atomic charges were added. The docking grids used were relatively large cubes ($22.5 \times 22.5 \times 22.5$ Å^3), centered around the binding site to provide enough space for the ligand to move and rotate freely in and out of the binding site. The 3D structures of the DAG-lactones were calculated with CORINA[57] and further optimized with the semiempirical quantum mechanics program Amsol 6.1.1 using the AM1 Hamiltonian.[58] Partial atomic charges were CM1 charges calculated with Amsol. For phorbol-13-acetate, the 3D structure was taken from the crystal structure without further minimization, and partial charges were calculated using the Gasteiger−Marsili method.[59] Nonpolar hydrogens were united with their covalently bonded carbon atoms. For each ligand, the docking simulation was repeated 100 times, starting each time from a different position and orientation chosen randomly.

Grids were calculated using the default force field parameters generated by the script *gen-gpf*. The spacing is 0.25 Å, and the number of points were 90 in each dimension. The grid center coordinates were 9.460, 26.699, and 21.364 Å relative to the coordinates of the PDB file 1ptr. For the dockings, we used the default intramolecular force field parameters generated by the script *gen-dpf*, except for hydrogen bonds with oxygens which were modeled explicitly by the directional Lennard-Jones 12-10 potential. The internal energy of the ligands included the electrostatic interactions. The initial coordinates were set to 14.6, 20.7, and 35.5 Å (inside the protein) to force the program to select randomly a different configuration of the ligand for each run. The simulated annealing RT factor had initially a value of 2.00 cal/mol and decreased linearly to 0 during 100 Monte Carlo cycles with a maximum of 30 000 accepted and 30 000 rejected steps per cycle. Each new cycle began with the state of minimum energy from the previous annealing cycle. Initial and final values per Monte Carlo step for the maximum translation, quaternion rotation, and torsion were, respectively, 0.2 and 0.1 Å, 5.00 and 1.00 Å, and 10.00 and 1.00 Å. Clustering of the docked conformations was performed with a tolerance of 1 Å.

Analysis of Inhibition of [^{3}H]PDBU Binding by Non-radioactive Ligands. Enzyme−ligand interactions were analyzed by competition with [^{3}H]PDBU binding essentially as described previously with the single isozyme PK-Cα.[24−26,42] This recombinant isozyme was expressed in the baculovirus system and was isolated as described in ref 60. The ID_{50} values were determined from the competition curves, and the corresponding K_i values for the ligands were calculated from the ID_{50} values as described before.[42] Values represent the mean ± standard error (three determinations).

Analysis of PK-Cα Activation. PK-C activation was determined by measuring the transfer of ^{32}P from [γ-32P]ATP to PK-Cα pseudosubstrate peptide as a function of activating ligand. PK-Cα was incubated in a total volume of 50 μL in the presence of 50 mM Tris-Cl, pH 7.4, 7.5 mM magnesium acetate, 0.1 mM $CaCl_2$, 0.25 mg/mL bovine serum albumin, 50 μM ATP, 1 μCi [γ-32P]ATP (specific activity > 5000 Ci/mmol), 200 nM PK-Cα pseudosubstrate peptide, 100 μg/mg phospholipid (80% phosphatidylcholine/20% phosphatidylserine), and activating ligand dissolved in dimethyl sulfoxide (final concentration of dimethyl sulfoxide was < 0.2%). Incubation was for 10 min at 30 °C. The reaction was then stopped by

chilling to 0 °C, and a 25 μL aliquot was spotted onto Whatman P81 ion exchange paper to bind the phosphorylated peptide. The paper was washed three times in 0.5% phosphoric acid and three times in water to remove unbound ATP. Bound radioactivity was then measured in a scintillation counter. Background activity was measured in the absence of PK-Cα. Maximal stimulation was determined in the presence of 1 μM PMA. Under the conditions of the assay, the response was linear with the amount of PK-Cα. Dose−response curves were determined from 8 to 10 concentrations of ligand. In each experiment, activation at each concentration of ligand was determined in duplicate. ED_{50} values (doses yielding half-maximal stimulation) represent the mean of three independent experiments, unless otherwise indicated.

Analysis of Inhibition of EGF Binding. Inhibition of binding of epidermal growth factor (EGF) to C3H10T1/2 cells was determined as follows. Cells were plated at 2.5×10^5 cells/well (6 plate well) on day 1 in Eagle's minimum essential medium containing 10% fetal bovine serum, 2 mM glutamine, and 100 μg/mL gentamicin and incubated at 37 °C in a humidified atmosphere containing 5% CO_2. Two days later, cells were washed with Eagle's minimum essential medium and then treated with Eagle's minimum essential medium containing 1 mg/mL bovine serum albumin, 25 mM BES buffer (*N*,*N*-bis[2-hydroxyethyl]-2-aminoethanesulfonic acid), pH 7.2, and dimethyl sulfoxide or compound dissolved in dimethyl sulfoxide for 60 min at 37 °C (final dimethyl sulfoxide concentration < 0.1%). Cells were then placed on ice and washed three times with cold Eagle's minimum essential medium. Fresh Eagle's minimum essential medium containing bovine serum albumin, BES buffer, and compound, together with 0.1 μCi/well ^{125}I-EGF in the absence or presence of 1 μg/well of nonradioactive EGF. The cells were incubated for 5 h in the cold room. Then, 100 μL supernatant was removed and radioactivity measured to determine total activity per well. The cells were washed three times with cold Eagle's minimum essential medium. The cells were finally lysed in 1 mL of 0.2 N NaOH, and aliquots of each lysate were counted in a scintillation counter to determine bound radioactivity. The average protein value was determined for 6 wells receiving the same treatment as controls without ^{125}I-EGF. Control activity was determined in the absence of added ligand. Background was determined in the presence of 1 μg/well of nonradioactive EGF. Dose−response curves were typically determined from six concentrations of ligand. In each experiment, inhibition at each concentration of ligand was determined in triplicate. ID_{50} values (doses yielding half-maximal inhibition) represent the mean of three independent experiments, unless otherwise indicated.

General Experimental Procedures. All chemical reagents were commercially available. Melting points were determined on a MelTemp II apparatus, Laboratory Devices, USA, and are uncorrected. Silica gel chromatography was performed on silica gel 60, 230−400 mesh (E. Merck). Proton and ^{13}C NMR spectra were recorded on a Bruker AC-250 instrument at 250 and 62.9 MHz, respectively. Spectra were 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 FTIR, and specific rotations were measured in a Perkin-Elmer model 241 polarimeter. Optical rotations were recorded on a Perkin-Elmer model 241 polarimeter. Positive-ion fast-atom bombardment mass spectra (FABMS) 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 analysis were performed by Atlantic Microlab, Inc., Atlanta, GA.

3,3-Diisopropyl-2-propenal [4-Methyl-3-(methylethyl)-pent-2-enal] (3). This compound was prepared according to the procedure of Denmark.[37]

3,3-Diisopropylpropionaldehyde [4-Methyl-3-(methylethyl)pentanal] (4). Compound **3** (6 g, 42.8 mmol) was dissolved in CH_2Cl_2 (55 mL) and hydrogenated at 41 psi in the presence of 10% Pd/C at ambient temperature. After 12 h the catalyst was filtered off with the aid of Celite, and the filtrate was concentrated. The crude product obtained was purified by flash column chromatography on silica gel with hexanes:Et_2O (98:2) as eluant to give **4** (5.81 g, 95%) as a clear oil: IR (neat) 2960−2875 and 1725 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$) δ 9.85 (m, 1 H, CHO), 2.36 (dd, 2 H, J = 5.2, 1.83 Hz, CH_2CHO), 1.83 m (2 H, 2 × (*H*CMe$_2$)$_2$), 1.75 (m, 1 H, *H*C(*i*-Pr)$_2$), 0.97 (d, 6 H, J = 6.6 Hz, HC(CH_3)$_2$), 0.88 (d, 6 H, J = 6.8 Hz, HC(CH_3)$_2$); ^{13}C NMR ($CDCl_3$) δ 203.43, 44.70, 42.86, 29.30, 21.40, 18.84. This compound was used directly in the following step.

3,3-Diisopropylpropionic Acid [4-Methyl-3-(methylethyl)pentanoic Acid] (5). Oxidation of this aldehyde was achieved quantitatively by a specific methodology.[61,62] A stirred solution of compound **4** (1 g, 7.0 mmol) in *t*-BuOH (9 mL) was treated with 2-methyl-2-butene (2 mL, 0.7 mmol), 1.3 equiv of $NaClO_2$ (1.37 g, 9.1 mmol), and an aqueous solution of $NaHPO_4$ (7 mL, 1.67 M). The oxidation was allowed to proceed at room temperature overnight, quenched with water (10 mL), acidified with concentrated HCl to pH 2, and extracted with Et_2O (3 × 30 mL). The combined organic extract was dried ($MgSO_4$), filtered, and concentrated to give the crude acid as a clear oil: IR (neat) 3050 (br), 2961, and 1708 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.31 (d, 2 H, J = 5.8 Hz, CH_2CO_2H), 1.84 (sextuplet, 2 H, 2 × *H*CMe$_2$)$_2$), 1.70 (m, 1 H, *H*C(*i*-Pr)$_2$), 1.00 (d, 6 H, J = 6.8 Hz, HC(CH_3)$_2$), 0.92 (d, 6 H, J = 6.6 Hz, HC(CH_3)$_2$); ^{13}C NMR ($CDCl_3$) δ 181.54, 46.55, 32.83, 29.32, 21.26, 18.64. This compound was used directly in the following step.

3,3-Diisopropylpropionic Acid Chloride [4-Methyl-3-(methylethyl)pentanoyl Chloride] (6). Treatment of a stirred solution of the crude acid (ca. 7 mmol) in Et_2O (14 mL) with oxalyl chloride (1 mL, 11.5 mmol) and pyridine (10 μL) at 0 °C for 1 h, and then at room temperature for 48 h, afforded the crude acyl chloride after removal of all the volatiles: IR (neat) 2962 and 1802 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.86 (d, 2 H, J = 5.1 Hz, CH_2COCl), 1.84 (m, 3 H, *H*C(*H*C(Me)$_2$)$_2$), 1.10 (d, 6 H, J = 6.6 Hz, HC(CH_3)$_2$), 0.92 (d, 6 H, J = 6.6 Hz, HC(CH_3)$_2$; ^{13}C NMR ($CDCl_3$) δ 174.51, 47.32, 46.50, 29.19, 21.18, 18.67. This compound was distilled once and used directly in all the acylation steps.

5,5-Bis[(2,2-dimethyl-1,1-diphenyl-1-silapropoxy)-methoxy]oxolan-2-one (33). The synthesis of this compound was reported earlier by Sharma et al.[24]

Standard Alkylation Procedure. A stirred solution of lactone **33** in THF (5 mL/mmol) was cooled to −78 °C under argon and treated dropwise with lithium diisopropylamide (1.4 equiv, 2 M solution in heptane/THF/ ethylbenzene). The mixture was stirred at −78 °C for 1.5−2 h, and a solution of the aldehyde (1.4 to 4 equiv) in THF (1 mL/mmol) was added dropwise to the lithium enolate at the same temperature. Stirring at −78 °C continued for 2 h, and the reaction was quenched by the slow addition of a saturated aqueous solution of ammonium chloride (1.25 mL/mmol) and then warmed to room temperature. The aqueous layer was extracted three times with Et_2O (4.15 mL/mmol), and the combined organic extracts were washed three times with water (2.50 mL/mmol), twice with brine (2.50 mL/mmol), dried ($MgSO_4$), and filtered. The filtrate was concentrated under vacuum to afford the crude alkylation reaction product, which was purified by flash column chromatography after eluting with the appropriate solvent. The obtained mixture of diastereoisomers was typically used directly in the following step without further purification.

Standard Mesylation−Olefination Procedure. A solution of the alkylation product in dichloromethane (10 mL/mmol) at 0 °C was treated with methanesulfonyl chloride (2 eq) and triethylamine (4 eq). This mixture was stirred at 0 °C for 30 min and then for 2 h at room temperature. 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU, 5 eq) was added at 0 °C, and the resulting solution was stirred overnight at ambient temperature. The reaction mixture was concentrated to a brown syrup and filtered through a pad of silica gel. The

filtrate was concentrated, and the residue was purified by flash column chromatography after eluting with the appropriate solvent.

Standard Desilylation Procedure. A stirred solution of the silylated compound in pyridine (2 mL/mmol) was treated dropwise with 4 equiv of HF/pyridine (70% HF solution in pyridine) while under argon at room temperature. Stirring was continued for 2 h, and the reaction was quenched by the addition of solid $NaHCO_3$. Filtration and concentration of the filtrate gave the deprotected residue which was purified by flash column chromatography after eluting with the appropriate solvent.

Standard Monoacylation Procedure. Method A. A mixture consisting of the corresponding 5,5-bis(hydroxymethyl)-oxolan-2-one (17 mL/mmol) dissolved in toluene, molecular sieves 4 Å, and dibutyltin oxide (1.5−2 eq) was heated to reflux for 1−2 h and then cooled to 0 °C before the addition of the corresponding acyl chloride (1.0 eq). The reaction mixture was stirred for 1 h at 0 °C and then quenched by the addition of an aqueous solution of potassium phosphate buffer (pH 7, 10 mL/mmol). After filtering off the solids, the aqueous layer was extracted three times with $CHCl_3$ (28 mL/mmol). The combined organic extract was dried ($MgSO_4$), filtered, and concentrated. The residue was purified by flash column chromatography with the appropriate solvent to give the monoacyl derivative as the major product and a smaller amount of the diacyl compound.

Method B. A solution of the 5,5-bis(hydroxymethyl)oxolan-2-one in CH_2Cl_2 (16.22 mL/mmol) at room temperature was treated with 2 equiv of pyridine and stirred for at least 1 h. The acyl chloride (1.0−1.5 eq) was added at 0 °C, and the mixture was allowed to stand at room temperature for 1 h. The reaction was then concentrated under vacuum and the residue purified by flash column chromatography with the appropriate solvent to give the monoacyl derivative as the major product.

5,5-Bis[(2,2-dimethyl-1,1-diphenyl-1-silapropoxy)-methyl]-3-[1-hydroxy-4-methyl-3-(methylethyl)pent-2-enyl]oxolan-2-one (34). Using the standard alkylation procedure starting from lactone **33** (15 g, 24.1 mmol) and 1.4 equiv of aldehyde **3**, compound **34** (17.21 g, 95%) was obtained as a mixture of diastereoisomers after column chromatography with hexanes:EtOAc (9:1).

5,5-Bis[(2,2-dimethyl-1,1-diphenyl-1-silapropoxy)-methyl]-3-[1-hydroxy-4-methyl-3-(methylethyl)pentyl]oxolan-2-one (35). Using the standard alkylation procedure starting from lactone **33** (8 g, 12.8 mmol) and 4 equiv of the aldehyde **4**, compound **35** (9.80 g, 99%) was isolated as a mixture of diastereoisomers after column chromatography with hexanes: EtOAc (9:1).

5,5-Bis[(2,2-dimethyl-1,1-diphenyl-1-silapropoxy)-methyl]-3-(hydroxyethyl)oxolan-2-one (36). Using the standard alkylation procedure starting from lactone **33** (15 g, 24.1 mmol) and 2 equiv of acetaldehyde (2.69 mL, 48.2 mmol), compound **36** (15.16 g, 94%) was isolated as a mixture of diastereoisomers after flash chromatography with hexanes: EtOAc (5:1).

5,5-Bis[(2,2-dimethyl-1,1-diphenyl-1-silapropoxy)-methyl]-3-(hydroxybutyl)oxolan-2-one (37). Using the standard alkylation procedure starting from lactone **33** (10 g, 16.1 mmol) and 3 equiv of *n*-butyraldehyde (4.34 mL, 72.1 mmol), compound **37** (11.15 g, 99%) was isolated as a mixture of diastereoisomers after flash chromatography with hexanes: EtOAc (9:1 to 4:1).

5,5-Bis[(2,2-dimethyl-1,1-diphenyl-1-silapropoxy)-methyl]-3-(hydroxyhexyl)oxolan-2-one (38). Using the standard alkylation procedure starting from lactone **33** (10 g, 16.1 mmol) and 3 equiv of *n*-hexanal (5.90 mL, 49.1 mmol), compound **38** (15.97 g, >90%) was isolated as a mixture of diastereoisomers after flash chromatography with hexanes: EtOAc (95:5 to 9:1).

5,5-Bis[(2,2-dimethyl-1,1-diphenyl-1-silapropoxy)-methyl]-3-(hydroxydecyl)oxolan-2-one (39). Using the standard alkylation procedure starting from lactone **33** (5 g, 8.0 mmol) and 1.5 equiv of *n*-decanal (2.3 mL, 12.2 mmol), compound **39** (>90%) was isolated as a mixture of diastereoisomers after flash chromatography with hexanes:EtOAc (95:5 to 9:1).

(*Z*)-5,5-Bis[(2,2-dimethyl-1,1-diphenyl-1-silapropoxy)-methyl]-3-[4-methyl-3-(methylethyl)pent-2-enylidene)-oxolan-2-one (40) and (*E*)-5,5-Bis[(2,2-dimethyl-1,1-diphenyl-1-silapropoxy)methyl]-3-[4-methyl-3-(methylethyl)-pent-2-enylidene)oxolan-2-one (41). Under the standard mesylation−olefination conditions starting from **34** (3.5 g, 4.6 mmol), three fractions were isolated after chromatography with hexanes:EtOAc (95:5). The first fraction corresponded to the *Z*-isomer (**40**, 0.903 g, 26%), the second fraction was a mixture of both *E*- and *Z*-isomers (0.164 g, 4%), and the third fraction was the *E*-isomer (**41**, 2.06 g, 60%).

Compound 40: oil; IR ($CHCl_3$) 2961, 1744 (C=O), 1632 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.46−7.70 (m, 21 H, Ph, >C=CHC*H*=C(*i*-Pr)$_2$), 7.06 (dd, 1 H, J = 12.2, 1.2 Hz, >C=C*H*CH=C(*i*-Pr)$_2$), 3.79 (m, 4 H, CH_2OSi), 3.20 (heptuplet, 1 H, J = 6.8 Hz, C*H*Me$_2$), 2.97 (d, 2 H, J = 2.4 Hz, H-4$_{a,b}$), 2.56 (heptuplet, 1 H, J = 6.8 Hz, C*H*Me$_2$), 1.17−1.19 (2 d, 12 H, J = 6.8 Hz, HC(C*H*$_3$)$_2$), 1.07 (s, 18 H,*t*-Bu); ^{13}C NMR ($CDCl_3$) δ 169.58 (C=O), 163.93, 135.62, 135.58, 133.54, 132.94, 132.73, 129.74, 127.72, 121.63, 117.18, 84.29, 66.29, 34.19, 29.68, 29.56, 29.34, 26.65, 24.17, 21.21, 19.20. Anal. ($C_{47}H_{60}O_4Si_2$) C, H.

Compound 41: white solid; mp 118−119 °C; IR ($CHCl_3$) 2958, 1741 (C=O), 1639; ^1H NMR ($CDCl_3$) δ 7.46−7.69 (m, 21 H, Ph, >C=C*H*CH=C(*i*-Pr)$_2$), 5.99 (d, 1 H, J = 12.2 Hz, >C=CHC*H*=C(*i*-Pr)$_2$), 3.84 (AB d, 2 H, J = 10.7 Hz, C*H*HOSi), 3.77 (AB d, 2 H, J = 10.7 Hz, CH*H*OSi), 3.28 (heptuplet, 1 H, J = 6.8 Hz, C*H*Me$_2$), 2.92 (d, 2 H, J = 2.4 Hz, H-4$_{a,b}$), 2.61 (heptuplet, 1 H, J = 6.8 Hz, C*H*Me$_2$), 1.17−1.19 (2 d, 12 H, HC(C*H*$_3$)$_2$), 0.97−1.05 (2 s, 18 H, *t*-Bu); ^{13}C NMR ($CDCl_3$) δ 171.83 (C=O), 166.94, 135.57, 132.88, 132.72, 130.58, 129.77, 127.73, 124.16, 117.55 85.36, 66.31 (*C*H$_2$OSi), 30.78, 30.26 (C-4), 29.65, 26.66, 24.18, 21.11, 19.20. Anal. ($C_{47}H_{60}O_4Si_2$) C, H.

(*Z*)-5,5-Bis[(2,2-dimethyl-1,1-diphenyl-1-silapropoxy)-methyl]-3-[4-methyl-3-(methylethyl)pentylidene]oxolan-2-one (42) and (*E*)-5,5-Bis[(2,2-dimethyl-1,1-diphenyl-1-silapropoxy)methyl]-3-[4-methyl-3-(methylethyl)pentylidene]oxolan-2-one (43). Under the standard mesylation−olefination conditions starting from **35** (6.65 g. 8.7 mmol), two fractions were isolated after chromatography with mixtures of hexane:petroleum ether:Et_2O (80:16:4) and (70:24:6) as eluants. The first fraction corresponded to the *Z*-isomer (**42**, 2.21 g, 34%) followed by the *E*-isomer (**43**, 2.99 g, 46%).

Compound 42: solid; mp 66−67 °C; IR ($CHCl_3$) 3071−2958, 1757 (C=O), 1666, 1114 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.70 and 7.47 (m, 20 H, phenyl), 6.23 (pseudo t, 1 H, >C=C*H*CH$_2$CH(*i*-Pr)$_2$), 3.78 (s, 4 H, C*H*$_2$OSi), 2.91 (narrow m, 2 H, H-4$_{a,b}$), 2.80 (m, 2 H, >C=CHC*H*$_2$CH(*i*-Pr)$_2$), 1.86 (m, 2 H, C*H*Me$_2$), 1.60 (m, 1 H, *H*C(*i*-Pr)$_2$), 1.09 (s, 18 H, *t*-Bu), 0.99 (d, 6 H, J = 6.8 Hz, HC(C*H*$_3$)$_2$), 0.94 (d, 6 H, J = 6.8 Hz, HC(C*H*$_3$)$_2$); ^{13}C NMR ($CDCl_3$) δ 169.34, 145.55, 135.63, 135.58, 129.78, 127.75, 132.94, 132.76, 124.30, 84.28, 65.93, 51.15, 33.03, 29.31, 26.71, 26.07, 21.64, 19.50, 19.24. Anal. ($C_{47}H_{62}O_4Si_2$) C, H.

Compound 43: solid; mp 68−69 °C; IR ($CHCl_3$) 3071−2858, 1761 (C=O), 1676, 1114 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.69 and 7.47 (m, 20 H, phenyl), 6.85 (m, 1 H, >C=C*H*CH$_2$−CH(*i*-Pr)$_2$), 3.82 (AB d, 2 H, J = 10.7 Hz, C*H*HOSi), 3.76 (AB d, 2 H, J = 10.7 Hz, CH*H*OSi), 2.86 (br s, 2H, H-4$_{a,b}$), 2.18 (pseudo t, 2 H, J = 7.1, 6.1 Hz, >C=CHC*H*$_2$CH(*i*-Pr)$_2$), 1.88 (m, 2 H, C*H*Me$_2$), 1.30 (m, 1 H, *H*C(*i*-Pr)$_2$), 1.27 (s, 18 H, *t*-Bu), 0.99 (d, 6 H, J = 6.6 Hz, HC(C*H*$_3$)$_2$), 0.94 (d, 6 H, J = 6.8 Hz, HC(C*H*$_3$)$_2$); ^{13}C NMR δ 170.58, 141.59, 135.63, 135.57, 129.82, 127.77, 132.91, 132.93, 126.62, 85.18, 66.10, 50.21, 29.66, 29.15, 28.44, 26.68, 21.59, 19.43, 19.22. Anal. ($C_{47}H_{62}O_4Si_2$) C, H.

(*Z*)-5,5-Bis[(2,2-dimethyl-1,1-diphenyl-1-silapropoxy)-methyl]-3-ethylideneoxolan-2-one (44) and (*E*)-5,5-Bis-[(2,2-dimethyl-1,1-diphenyl-1-silapropoxy)methyl]-3-eth-ylideneoxolan-2-one (45). Under the standard mesylation−olefination conditions starting from **36** (13.26 g, 19.9 mmol), three fractions were isolated after chromatography with mixtures of hexane:EtOAc (95:5 to 3:1) as eluant. The first fraction corresponded to the *Z*-isomer (**44**, 3.71 g, 29%) followed

by a mixture of *Z*- and *E*-isomers (1.97 g, 15%) and a third fraction of the *E*-isomer (**45**, 6.72 g, 52%).

Compound 44: solid; mp 113−114 °C; IR ($CHCl_3$) 1750 (C=O), 1674 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.69 and 7.48 (m, 20 H, Ph), 6.28 (qt, 1H, J = 7.3, 2.2 Hz, >C=C*H*CH_3), 3.81 (AB d, 2 H, J = 10.6 Hz, C*H*HOSi), 3.75 (AB d, 2H, J = 10.6 Hz, CH*H*OSi), 2.90 (m, 2 H, H-$4_{a,b}$), 2.25 (dt, 3 H, J = 7.3, 2.2 Hz, >C=CHC*H*$_3$), 1.09 (s, 18 H, *t*-Bu); ^{13}C NMR ($CDCl_3$) δ 169.36, 137.22, 135.50, 135.46, 132.77, 132.59, 129.69, 127.66, 126.41, 84.35, 66.10, 33.32, 26.66, 19.21, 13.93. Anal. ($C_{40}H_{48}O_4Si_2$) C, H.

Compound 45: solid; mp 128−129 °C; IR ($CHCl_3$) 1751 (C=O), 1684 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.68 and 7.48 (m, 20 H, Ph), 6.84 (m, 1H, >C=C*H*CH_3), 3.83 (AB d, 2 H, J = 10.7 Hz, C*H*HOSi), 3.77 (AB d, 2H, J = 10.7 Hz, CH*H*OSi), 2.83 (br s, 2 H, H-$4_{a,b}$), 2.25 (br d, 3 H, J = 7.1 Hz, >C=CHC*H*$_3$), 1.08 (s, 18 H, *t*-Bu); ^{13}C NMR (CDCl3) δ 170.16, 135.47, 135.43, 134.09, 132.74, 132.51, 129.69, 128.59, 127.64, 85.11, 66.12, 29.64, 26.62, 19.18, 15.53. Anal. ($C_{40}H_{48}O_4Si_2$) C, H.

***(Z)*-5,5-Bis[(2,2-dimethyl-1,1-diphenyl-1-silapropoxy)methyl]-3-butylideneoxolan-2-one (46) and *(E)*-5,5-Bis[(2,2-dimethyl-1,1-diphenyl-1-silapropoxy)methyl]-3-butylideneoxolan-2-one (47).** Under the standard mesylation−olefination conditions starting from **37** (10.43 g, 15.0 mmol), two fractions were isolated after chromatography with a mixture of hexane:petroleum ether:Et_2O (8):16:4) as eluant. The first fraction corresponded to the *Z*-isomer (**46**, 2.44 g, 24%) followed by the *E*-isomer (**47**, 5.78 g, 57%).

Compound 46: solid; mp 70−75 °C; IR (neat) 3061−2990, 1744 (C=O), 1678 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.70 and 7.47 (m, 20 H, Ph), 6.20 (m, 1H, >C=C*H*$(CH_2)_2CH_3$), 3.79 (AB d, 2 H, J = 10.8 Hz, C*H*HOSi), 3.76 (AB d, 2H, J = 10.8 Hz, CH*H*OSi), 2.91 (br d, 2 H, J = 2.0 Hz, H-$4_{a,b}$), 2.77 (br q, 2 H, J = 7.3, >C=CHCH_2CH_2CH_3), 1.53 (multiplet, 2 H, >C=CHCH_2CH_2-CH_3), 1.09 (s, 18 H, *t*-Bu), 1.02 (br t, 3 H, >C=CH$(CH_2)_2$CH_3); ^{13}C NMR ($CDCl_3$) δ 169.19, 142.89, 135.49, 135.45, 135.37, 132.77, 132.59, 129.68, 127.76, 127.74, 127.64, 125.49, 84.30, 66.01, 33.13, 29.47, 26.68, 22.34, 19.21, 13.79. Anal. ($C_{42}H_{52}O_4$-Si_2·0.5H_2O) C, H.

Compound 47: solid; mp 72−73 °C; IR (neat) 3116−2906, 1740 (C=O), 1632 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.70 and 7.46 (m, 20 H, Ph), 6.79 (m, 1H, >C=C*H*$(CH_2)_2CH_3$), 3.82 (AB d, 2 H, J = 10.8 Hz, C*H*HOSi), 3.76 (AB d, 2H, J = 10.8 Hz, CH*H*OSi), 2.84 (br s, 2 H, H-$4_{a,b}$), 2.22 (q, 2 H, J = 7.3 Hz, >C=CHCH_2-CH_2CH$_3$), 1.59 (multiplet, 2 H, >C=CHCH_2CH_2CH$_3$), 1.07 (br s, 18 H, *t*-Bu), 1.04 (br t, 3 H, >C=CH$(CH_2)_2$CH_3); ^{13}C NMR ($CDCl_3$) δ 170.38, 139.36, 135.48, 135.44, 132.75, 132.52, 129.69, 127.65, 127.58, 85.12, 66.11, 32.09, 29.67, 26.65, 21.43, 19.19, 13.89. Anal. ($C_{42}H_{52}O_4Si_2$·0.29H_2O) C, H.

***(Z)*-5,5-Bis[(2,2-dimethyl-1,1-diphenyl-1-silapropoxy)methyl]-3-hexylideneoxolan-2-one (48) and *(E)*-5,5-Bis[(2,2-dimethyl-1,1-diphenyl-1-silapropoxy)methyl]-3-hexylideneoxolan-2-one (49).** Under the standard mesylation−olefination conditions starting from **38** (11.60 g, 16.1 mmol), two fractions were isolated after chromatography with a mixture of hexanes:EtOAc (98:2 and 95:5) as eluant. The first fraction corresponded to the *Z*-isomer (**48**, 2.69 g, 24%) followed by the *E*-isomer (**49**, 8.26 g, 73%).

Compound 48: solid; mp 90−92 °C; IR ($CHCl_3$) 3019−2862, 1744 (C=O), 1668 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.71 and 7.46 (m, 20 H, Ph), 6.20 (br t, 1H, $J \approx$ 7.6 Hz, >C=C*H*$(CH_2)_4CH_3$), 3.79 (AB d, 2 H, J = 10.8 Hz, C*H*HOSi), 3.76 (AB d, 2H, J = 10.8 Hz, CH*H*OSi), 2.91 (br d, 2 H, $J \approx$ 2.0 Hz, H-$4_{a,b}$), 2.72 (br q, 2 H, $J \approx$ 7.3 Hz, >C=CHC$H_2$$(CH_2)_3CH_3$), 1.41 (m, 6 H, >C=CHC$H_2$$(CH_2)_3CH_3$), 1.09 (s, 18 H, *t*-Bu), 1.02 (br t, 3 H, >C=CH$(CH_2)_4$CH_3); ^{13}C NMR ($CDCl_3$) δ 169.19, 143.18, 135.47, 135.42, 135.34, 132.75, 132.56, 129.64, 127.60, 125.23, 84.28, 65.98, 33.09, 31.41, 28.77, 27.48, 26.64, 22.46, 19.18, 13.96. Anal. ($C_{44}H_{56}O_4Si_2$·0.7H_2O) C, H.

Compound 49: oil; IR (neat) 3061−2862, 1761 (C=O), 1680 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.70 and 7.46 (m, 20 H, Ph), 6.79 (m, 1H, >C=C*H*$(CH_2)_4CH_3$), 3.82 (AB d, 2 H, J = 10.7 Hz, C*H*HOSi), 3.75 (AB d, 2H, J = 10.7 Hz, CH*H*OSi), 2.83 (br s, 2 H, H-$4_{a,b}$), 2.23 (br q, 2 H, $J \approx$ 7.3 Hz, >C=CHC$H_2$$(CH_2)_3$-$CH_3$), 1.56 and 1.40 (multiplets, 6 H, >C=CHC$H_2$$(CH_2)_3CH_3$), 1.08 (s, 18 H, *t*-Bu), 0.98 (br t, 3 H, >C=CH$(CH_2)_4$CH_3); ^{13}C NMR ($CDCl_3$) δ 170.40, 139.62, 135.46, 135.42, 132.74, 132.48, 129.67, 127.62, 127.35, 85.11, 66.08, 31.46, 30.06, 29.61, 27.74, 26.62, 22.41, 19.17, 13.91. Anal. ($C_{44}H_{56}O_4Si_2$) C, H.

***(Z)*-5,5-Bis[(2,2-dimethyl-1,1-diphenyl-1-silapropoxy)methyl]-3-decylideneoxolan-2-one (50) and *(E)*-5,5-Bis[(2,2-dimethyl-1,1-diphenyl-1-silapropoxy)methyl]-3-decylideneoxolan-2-one (51).** Under the standard mesylation−olefination conditions starting from **39** (5.33 g, 6.8 mmol), two fractions were isolated after chromatography with a mixture of hexanes:EtOAc (97:3 and 95:5) as eluant. The first fraction corresponded to the *Z*-isomer (**50**, 1.02 g, 20%) followed by the *E*-isomer (**51**, 3.39 g, 65%).

Compound 50: oil; IR (neat) 3071−2856, 1759 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.71 and 7.41 (m, 20 H, Ph), 6.20 (br t, 1H, $J \approx$ 7.5 Hz, >C=C*H*$(CH_2)_8CH_3$), 3.78 (AB q, 4 H, J = 10.7 Hz, 2 × C*H*HOSi), 2.91 (br d, 2 H, $J \approx$ 1.7 Hz, H-$4_{a,b}$), 2.77 (br q, 2 H, $J \approx$ 7.0 Hz, >C=CHC$H_2$$(CH_2)_7CH_3$), 1.35−1.50 (m, 14 H, >C=CHCH_2(CH_2)$_7$CH$_3$), 1.09 (s, 18 H, 2 × *t*-Bu), 0.97 (br t, 3 H, >C=CH$(CH_2)_8$CH_3). Anal. ($C_{48}H_{64}O_4Si_2$) C, H.

Compound 51: oil, IR (neat) 3071−2857, 1763 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.71 and 7.41 (m, 20 H, Ph), 6.79 (m, 1 H, >C=C*H*$(CH_2)_8CH_3$), 3.79 (AB q, 4 H, J = 10.7 Hz, 2 × CH_2-OSi), 2.83 (br s, 2 H, H-$4_{a,b}$), 2.23 (br q, 2 H, $J \approx$ 7.0 Hz, >C=CHC$H_2$$(CH_2)_7CH_3$), 1.60 and 1.35 (multiplets, 14 H, >C=CHCH_2(CH_2)$_7$CH$_3$), 1.08 (s, 18 H, 2 × *t*-Bu), 0.97 (br t, 3 H, >C=CH$(CH_2)_8$CH_3). Anal. ($C_{48}H_{64}O_4Si_2$) C, H.

***(Z)*-5,5-Bis(hydroxymethyl)-3-[4-methyl-3-(methylethyl)pent-2-enylidene]oxolan-2-one (52).** Starting from **40** (0.795 g, 1.2 mmol), the standard desilylation conditions were applied. After chromatography with hexanes:EtOAc (1:1), compound **52** (0.256 g, 80%) was obtained as white flakes: mp 132−133 °C; IR ($CHCl_3$) 3599 (OH), 3414 (br OH), 3015−2966, 1740 (C=O), 1629 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.36 (broad d, 1 H, J = 11.76 Hz, >C=CHC*H*=C(*i*-Pr)$_2$), 7.15 (d, 1 H, J = 12.2 Hz, >C=C*H*CH=C(*i*-Pr)$_2$), 3.85 (AB d, 2 H, J = 12.2 Hz, C*H*HOH), 3.79 (AB d, 2H, J = 12.2 Hz, CH*H*OH), 3.20 (heptuplet, 1 H, J = 6.8 Hz, C*H*Me$_2$), 2.93 (d, 2H, J = 1.7 Hz, H-$4_{a,b}$), 2.59 (broad s, 2 H, OH), 2.56 (heptuplet, 1 H, J = 6.7 Hz, C*H*Me$_2$) 1.18−1.15 (2 d, 12 H, J = 6.8 Hz, HC(CH_3)$_2$); ^{13}C NMR ($CDCl_3$) δ 169.56 (C=O), 166.09, 135.94, 119.61, 116.89, 84.22, 65.13 (*C*H_2OH), 33.04 (C-4), 29.62, 29.55, 29.48, 24.18, 21.17; FABMS (*m*/*z*, relative intensity) 269 (MH^+, 100). Anal. ($C_{15}H_{24}O_4$) C, H.

***(E)*-5,5-Bis(hydroxymethyl)-3-[4-methyl-3-(methylethyl)pent-2-enylidene]oxolan-2-one (53).** Starting from **41** (1.33 g, 2.0 mmol) the standard desilylation conditions were applied. After chromatography with hexanes:EtOAc (1:1), compound **53** (0.554 g, 100%) was obtained as white needles: mp 131−132 °C; IR ($CHCl_3$) 3599 (OH), 3414 (br OH), 3015, 1742 (C=O), 1634 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.61 (broad d, 1 H, J = 12.2 Hz, >C=C*H*CH=C(*i*-Pr)$_2$), 5.99 (d, 1 H, J = 12.2 Hz, >C=CHC*H*=C(*i*-Pr)$_2$), 3.89 (AB d, 2 H, J = 12.2 Hz, C*H*HOH), 3.81 (AB d, 2H, J = 12.2 Hz, CH*H*OH), 3.26 (heptuplet, 1 H, J = 6.8 Hz, C*H*Me$_2$), 2.92 (d, 2H, J = 2.2 Hz, H$4_{a,b}$), 2.59 (heptuplet, 1 H, J = 6.8 Hz, C*H*Me$_2$), 2.56 (broad s, 2 H, OH), 1.16 (d, 12 H, J = 6.8 Hz, HC(CH_3)$_2$); ^{13}C NMR ($CDCl_3$) δ 172.04 (C=O), 168.70, 132.71, 122.35, 117.48, 85.36 (C-5), 65.13 (*C*H_2OH), 30.51, 29.68, 29.72 (C-4), 24.09, 21.07; FABMS (*m*/*z*, relative intensity) 269 (MH^+, 100). Anal. ($C_{15}H_{24}O_4$·0.3H_2O) C, H.

***(Z)*-5,5-Bis(hydroxymethyl)-3-[4-methyl-3-(methylethyl)pentylidene]oxolan-2-one (54).** Starting from **42** (4.05 g, 5.4 mmol) the standard desilylation conditions were applied. After chromatography with mixtures hexanes:EtOAc (1:1) and (1:2), compound **54** (1.37 g, 93%) was obtained as a clear oil: IR (neat) 3432 (OH), 2958−2871, 1725 (C=O), 1669 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.34 (tt, 1 H, J = 7.3, 1.9 Hz, >C=C*H*CH_2−CH(*i*-Pr)$_2$), 3.83 (AB d, 2 H, J = 12.1 Hz, C*H*HOH), 3.77 (AB d, 2 H, J = 12.1 Hz, CH*H*OH), 2.85 (br d, 2H, J = 1.9 Hz, H-$4_{a,b}$), 2.78 (ddd, 1 H, J = 7.3, 5.9, 1.9 Hz, >C=CHCH_2−CH-(*i*-Pr)$_2$), 2.50 (br s, 2 H, OH), 1.85 (heptuplet, 2 H, C*H*Me$_2$), 1.19 (quintuplet, 1 H, C*H*(*i*-Pr)$_2$), 0.98−0.91 (2d, 12 H, J = 6.8

Hz, HC(CH_3)$_2$); ^{13}C NMR ($CDCl_3$) δ 169.40, 146.91, 122.72, 84.22, 63.96, 50.50, 32.34, 28.73, 25.72, 21.05, 18.86; FABMS (*m*/*z*, relative intensity) 271 (MH^+, 100). Anal. ($C_{15}H_{26}O_4 \cdot 0.16H_2O$) C, H.

(*E*)-5,5-Bis(hydroxymethyl)-3-[4-methyl-3-(methylethyl)pentylidene]oxolan-2-one (55). Starting from **43** (2.06 g, 7.6 mmol) the standard desilylation conditions were applied. After chromatography with mixtures hexanes:EtOAc (1:1), (1:2), and (1:3), compound **55** (1.97 g, 96%) was obtained as a clear oil: IR ($CHCl_3$) 3406 (OH), 2958−2873, 1739 (C=O), 1674 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.84 (m, 1 H, >C=C*H*CH$_2$CH(*i*-Pr)$_2$), 3.83 (AB d, 2 H, J = 12.1 Hz, C*H*HOH), 3.76 (AB d, 2 H, J = 12.1 Hz, CH*H*OH), 3.65 (m, 2 H, OH), 2.77 (br s, 2H, H-4$_{a,b}$), 2.19 (pseudo t, 2 H, >C=CHC*H*$_2$CH(*i*-Pr)$_2$), 1.83 (heptuplet, 2 H, C*H*Me$_2$), 1.27 (quintuplet, 1 H, *H*C(*i*-Pr)$_2$), 0.97 (d, 6 H, J = 6.8 Hz, HC(CH_3)$_2$), 0.91 (d, 6H, J = 6.6 Hz, HC(CH_3)$_2$); ^{13}C NMR ($CDCl_3$) δ 170.61, 143.13, 124.92, 85.16, 64.09, 49.69, 29.02, 28.65, 28.09, 21.03, 18.77; FABMS (*m*/*z*, relative intensity) 271 (MH^+, 100). Anal. ($C_{15}H_{26}O_4 \cdot 0.2H_2O$) C, H.

(*Z*)-5,5-Bis(hydroxymethyl)-3-ethylideneoxolan-2-one (56). Starting from **44** (2.59 g, 4.0 mmol) the standard desilylation conditions were applied. After chromatography with mixtures hexanes:EtOAc (1:2) and (1:5), 0.62 g (90%) of a ca. 1:1 mixture of *E*- and *Z*-isomers (**56** and **57**) was obtained: IR (Nujol) 3365 (OH), 1740 (C=O), 1764 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.75 and 6.39 (multiplets, 1 H, >C=C*H*CH$_3$); FABMS (*m*/*z*, relative intensity) 173 (MH^+, 100).

(*E*)-5,5-Bis(hydroxymethyl)-3-ethylideneoxolan-2-one (57). Starting from **45** (5.39 g, 8.3 mmol) the standard desilylation conditions were applied. After chromatography with mixtures hexanes:EtOAc (1:2) and (1:5), compound **57** (1.43 g, 100%) was obtained as a white solid: mp 105−106 °C; IR (Nujol) 3430−3354 (OH), 1732 (C=O), 1674 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.75 (m, 1 H, >C=C*H*CH$_3$), 3.72 (AB d, 2 H, J = 12.0 Hz, 2 × C*H*HOH), 3.64 (AB d, 2 H, J = 12.0 Hz, 2 × CH*H*OH), 2.83 (br m, 2 H, H-4$_{a,b}$), 1.92 (dt, 3H, J = 7.1, 1.7 Hz, >C=CHCH_3); ^{13}C NMR (CD_3OD) δ 172.83, 136.34, 130.30, 87.67, 65.34, 29.84, 15.84; FABMS (*m*/*z*, relative intensity) 173 (MH^+, 100). Anal. ($C_8H_{12}O_4$) C, H.

(*Z*)-5,5-Bis(hydroxymethyl)-3-butylideneoxolan-2-one (58). Starting from **46** (1.05 g, 1.6 mmol) the standard desilylation conditions were applied. After chromatography with mixtures hexanes:EtOAc (1:2) and (1:3), compound **58** (0.22 g, 70%) was obtained as an oil: IR (neat) 3395 (OH), 2958−2870, 1738 (C=O), 1667 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.31 (m, 1 H, >C=C*H*(CH$_2$)$_2$CH$_3$), 3.82 (AB d, 2 H J = 12.2 Hz, C*H*HOH), 3.76 (AB d, 2H, J = 12.1 Hz, CH*H*OH), 3.34 (s, 2 H, CH$_2$O*H*), 2.85 (br d, 2 H, J = 2.0 Hz, H-4$_{a,b}$), 2.73 (br q, 2 H, J ∼ 7.3 Hz, >C=CHC*H*$_2$CH$_2$CH$_3$), 1.53 (sextuplet, 2 H, J ∼ 7.3 Hz, >C=CHCH$_2$C*H*$_2$CH$_3$), 1.01 (t, 3 H, J ∼ 7.3 Hz, >C=CH(CH$_2$)$_2$CH_3); ^{13}C NMR ($CDCl_3$) δ 169.95, 145.02, 124.52, 84.87, 64.37, 32.54, 29.52, 22.17, 13.64; FABMS (*m*/*z*, relative intensity) 201(MH^+, 100). Anal. ($C_{10}H_{16}O_4 \cdot 0.13H_2O$) C, H.

(*E*)-5,5-Bis(hydroxymethyl)-3-butylideneoxolan-2-one (59). Starting from **47** (4.5 g, 6.7 mmol) the standard desilylation conditions were applied. After chromatography with mixtures hexanes:EtOAc (1:2), (1:3), and (1:5), compound **59** (1.24 g, 93%) was obtained as a white solid: mp 71−72 °C; IR (Nujol) 3382 (OH), 3017−2873, 1743 (C=O), 1778 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.80 (m, 1 H, >C=C*H*(CH$_2$)$_2$CH$_3$), 3.85 (AB d, 2 H J = 12.1 Hz, C*H*HOH), 3.76 (AB d, 2H, J = 12.1 Hz, CH*H*OH), 3.16 (s, 2 H, CH$_2$O*H*), 2.79 (br s, 2 H, H-4$_{a,b}$), 2.23 (br q, 2 H, J ∼ 7.3 Hz, >C=CHC*H*$_2$CH$_2$CH$_3$), 1.59 (sextuplet, 2 H, J ∼ 7.3 Hz, >C=CHCH$_2$C*H*$_2$CH$_3$), 1.02 (t, 3 H, J ∼ 7.3 Hz, >C=CH(CH$_2$)$_2$CH_3); ^{13}C NMR ($CDCl_3$) δ 171.19, 141.47, 126.69, 85.82, 64.53, 32.09, 29.26, 21.31, 13.77; FABMS (*m*/*z*, relative intensity) 201 (MH^+, 100). Anal. ($C_{10}H_{16}O_4$) C, H.

(*Z*)-5,5-Bis(hydroxymethyl)-3-hexylideneoxolan-2-one (60). Starting from **48** (2.29 g, 3.3 mmol) the standard desilylation conditions were applied. After chromatography with mixtures hexanes:EtOAc (1:1) and (1:2), compound **60** (0.66 g, 89%) was obtained as a clear oil: IR (neat) 3400 (OH), 2928−2863, 1739 (C=O), 1671 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.32 (tt, 1 H, J = 7.7, 2.2 Hz, >C=C*H*(CH$_2$)$_4$CH$_3$), 3.84 (dd, 2 H, J = 12.0, 6.1 Hz, 2 × C*H*HOH), 3.78 (dd, 2 H, J = 12.0, 5.3 Hz, 2 × CH*H*OH), 2.86 (m, 2 H, >C=CHC*H*$_2$(CH$_2$)$_3$CH$_3$), 2.80 (m, 2 H, H-4$_{a,b}$), 1.40−1.50 (m, 6 H, >C=CHCH$_2$(C*H*$_2$)$_3$CH$_3$), 0.96 (m, 3 H, >C=CH(CH$_2$)$_4$CH_3); ^{13}C NMR ($CDCl_3$) δ 171.12, 141.83, 126.38, 85.72, 64.62, 31.40, 30.13, 29.26, 27.64, 22.34, 13.88; FABMS (*m*/*z*, relative intensity) 229 (MH^+, 100). This compound was used without further purification in the next acylation step.

(*E*)-5,5-Bis(hydroxymethyl)-3-hexylideneoxolan-2-one (61). Starting from **49** (8.00 g, 11.3 mmol) the standard desilylation conditions were applied. After chromatography with mixtures hexanes:EtOAc (1:1) and (1:2), compound **61** (2.16 g, 83%) was obtained as a clear oil: IR ($CHCl_3$) 3381 (OH), 2956−2860, 1747 (C=O), 1679 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.80 (m, 1 H, >C=C*H*(CH$_2$)$_4$CH$_3$), 3.85 (dd, 2 H, J = 12.0, 5.2 Hz, C*H*HOH), 3.76 (dd, 2 H, J = 12.0, 4.7 Hz, CH*H*OH), 3.44 (br t, 2 H, CH$_2$O*H*), 2.77 (br s, 2 H, H-4$_{a,b}$), 2.24 (br q, 2 H, J ≈ 7.3 Hz, >C=CHC*H*$_2$(CH$_2$)$_3$CH$_3$), 1.37−1.54 (m, 6 H, >C=CHCH$_2$-(C*H*$_2$)$_3$CH$_3$), 0.96 (br t, 3 H, J ≈ 6.5 Hz, >C=CH(CH$_2$)$_4$CH_3); ^{13}C NMR ($CDCl_3$) δ 171.12, 141.83, 126.38, 85.72, 64.62, 31.40, 30.13, 29.26, 27.64, 22.34, 13.88; FABMS (*m*/*z*, relative intensity) 229 (MH^+, 100). This compound was used without further purification in the next acylation step.

(*Z*)-5,5-Bis(hydroxymethyl)-3-decylideneoxolan-2-one (62). Starting from **50** (4.89 g, 6.5 mmol) the standard desilylation conditions were applied. After chromatography with mixtures hexanes:EtOAc (2:3) and (1:4), compound **62** (1.77 g, 100%) was obtained as a clear oil: IR ($CHCl_3$) 3396 (OH), 2962−2855, 1742 (C=O), 1669 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.33 (tt, 1 H, J = 7.8, 2.2 Hz, 1 H, >C=C*H*(CH$_2$)$_8$CH$_3$), 3.82 (d AB q, 4 H J = 12.1, 6.4 Hz, 2 × C*H*$_2$OH), 2.88 (m, 2 H, H-4$_{a,b}$), 2.78 (m, 2 H, >C=CHC*H*$_2$(CH$_2$)$_7$CH$_3$), 2.12 (t, 1 H, J = 6.4 Hz, OH), 1.34−1.60 (m, 14 H, >C=CH(C*H*$_2$)$_7$CH$_3$), 0.96 (br t, 3 H, >C=CH(CH$_2$)$_8$CH_3); ^{13}C NMR ($CDCl_3$) δ 169.20, 145.76, 123.89, 84.22, 64.96, 32.60, 31.85, 31.81, 29.63, 29.44, 29.37, 29.29, 29.21, 28.99, 27.72, 22.61, 14.05; FABMS (*m*/*z*, relative intensity) 285 (MH^+, 100). Anal. ($C_{16}H_{28}O_4$) C, H.

(*E*)-5,5-Bis(hydroxymethyl)-3-decylideneoxolan-2-one (63). Starting from **51** (6.58 g, 8.6 mmol) the standard desilylation conditions were applied. After chromatography with mixtures hexanes:EtOAc (2:3) and (1:4), compound **63** (2.29 g, 93%) was obtained as a clear oil: IR ($CHCl_3$) 3408−3018 (OH), 2918−2853, 1747 (C=O), 1678 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.74 (m, 1 H, >C=C*H*(CH$_2$)$_8$CH$_3$), 3.85 (AB q, 4 H J = 12.1 Hz, 2 × C*H*$_2$OH), 2.71 (m, 2 H, H-4$_{a,b}$), 2.16 (br q, 2 H, J ≈ 7.3 Hz, >C=CHC*H*$_2$(CH$_2$)$_7$CH$_3$), 1.97 (br s, 1 H, OH), 1.46 (m, 2 H, >C=CHCH$_2$C*H*$_2$(CH$_2$)$_6$CH$_3$), 1.24 (m, 12 H, >C=CHCH$_2$(C*H*$_2$)$_6$CH$_3$), 0.86 (br t, 3 H, >C=CH(CH$_2$)$_8$CH_3); ^{13}C NMR ($CDCl_3$) δ 170.34, 142.26, 126.11, 84.49, 65.22, 31.82, 30.27, 29.45, 29.34, 29.23, 28.03, 22.68, 14.06; FABMS (*m*/*z*, relative intensity) 285 (MH^+, 100). Anal. ($C_{16}H_{28}O_4$) C, H.

5,5-Bis(hydromethyl)-3-[4-methyl-3-(methylethyl)pentyl]oxolane-2-one (64). A solution of **53** (0.220 g, 0.8 mmol) in 10 mL of ethanol was reduced in a Parr hydrogenator under 40 psi of hydrogen for 6 h in the presence of 10% Pd/C (0.022 g). The catalyst was filtered off, and the ethanol was evaporated to give a crude oil that was purified by flash column chromatography with hexanes:EtOAc (1:1 to 1:2) as eluant to give 0.224 mg (100%) of **64**: oil; IR (neat) 3388 (OH), 2956−2873 and 1747 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.84 (AB q, 2 H J = 12.2 Hz, C*HH*OH), 3.87 (AB q, 2H, J = 12.2 Hz, C*HH*OH), 3.20 (br s, 2 H, CH$_2$O*H*), 2.89 (ddd, 1 H, J = 14.0, 9.5, 4.2 Hz, H-3), 2.41 (dd, 1H, J = 12.9, 10.1 Hz, H-4$_a$), 1.75−2.00 (m, 5 H, H-4$_b$, CH_2CH_2CH(*i*-Pr)$_2$), 1.20−1.58 (m, 3 H, C*H*(C*H*Me$_2$)$_2$), 0.90 (m, 12 H, 2 × CH(CH_3)$_2$); ^{13}C NMR ($CDCl_3$) δ 179.36, 86.13, 64.59, 64.25, 49.50, 40.49, 31.71, 31.23, 24.85, 28.75, 28.57, 20.95, 20.81, 18.86, 18.65; FABMS (*m*/*z*, relative intensity) 273 (MH^+, 100). Anal. ($C_{15}H_{28}O_4$) C, H.

1,4-*cis*-{1-(Hydroxymethyl)-4-[4-methyl-3-(methylethyl)pentyl]-3-oxo-2-oxolanyl}methyl Hexanoate (65) and 1,4-*trans*-{1-(Hydroxymethyl)-4-[4-methyl-3-(methylethyl)pentyl]-3-oxo-2-oxolanyl}methyl Hexanoate (66). Standard monoacylation conditions (method A) starting from **64** (0.100 g, 0.37 mmol) were applied. After chromatography with

hexanes:EtOAc (3:1), some diacylated product was isolated (0.047 g, 27%), together with the 1,4-*cis*-monoacylated isomer **65** (0.033 g (24%) and the 1,4-*trans*-monoacylated isomer **66** (0.014 g, 10%).

Compound 65: oil; IR (neat) 3451 (OH), 2955−2870, 1773 and 1744 (C═O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.34 (AB d, 1 H J = 11.5 Hz, C*H*HOCO$(CH_2)_4CH_3$), 4.25 (d, 1 H, J = 11.5 Hz, CH*H*OCO$(CH_2)_4CH_3$), 3.85 (AB d, 1 H, J = 12.2 Hz, C*H*HOH), 3.68 (AB d, 1 H, J = 12.2 Hz, CH*H*OH), 2.85 (ddd, 1 H, J = 14.0, 9.8, 4.5 Hz, H-3), 2.45 (t, 2 H, J = 7.4 Hz, COC$H_2(CH_2)_3CH_3$), 2.40 (m, 1 H, H-4_a), 1.75−2.10 (m, 5 H, H-4_b, CH_2CH_2-CH(i-Pr)$_2$), 1.70 (m, 2 H, COCH_2C$H_2(CH_2)_4CH_3$), 1.25−1.60 (m, 7 H, CO$(CH_2)_2(CH_2)_2CH_3$, C*H*(C*H*Me$_2$)$_2$), 0.95 (m, 15 H, CO$(CH_2)_4CH_3$, 2 × CH(CH_3)$_2$); ^{13}C NMR ($CDCl_3$) δ 178.04, 173.19, 83.72, 65.28, 64.93, 49.99, 40.63, 33.98, 32.14, 31.79, 31.14, 29.25, 29.06, 25.31, 24.45, 22.21, 21.44, 21.29, 19.34, 19.14, 13.80; FABMS (m/z, relative intensity) 371 (MH^+, 62), 99 ($C_5H_{11}CO^+$, 99). Anal. ($C_{21}H_{38}O_5$) C, H.

Compound 66: oil; IR (neat) 3459 (OH), 2955−2870, 1774 and 1744 (C═O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.43 (AB d, 1 H J = 12.2 Hz, C*H*HOCO$(CH_2)_4CH_3$), 4.15 (d, 1 H, J = 12.2 Hz, CH*H*OCO$(CH_2)_4CH_3$), 3.80 (AB d, 1 H, J = 11.5 Hz, C*H*HOH), 3.70 (AB d, 1 H, J = 11.5 Hz, CH*H*OH), 2.86 (ddd, 1 H, J = 14.0, 10.0, 4.5 Hz, H-3), 2.55 (dd, 1 H, J = 12.94, 10.0 Hz, H-4_a), 2.45 (t, 2 H, J = 7.4 Hz, COC$H_2(CH_2)_3CH_3$), 1.65−2.10 (m, 7 H, H-4_b, CH_2CH_2CH(i-Pr)$_2$, COCH_2C$H_2(CH_2)_4CH_3$), 1.25−1.50 (m, 7 H, CO$(CH_2)_2(CH_2)_2CH_3$, C*H*(C*H*Me$_2$)$_2$), 0.95 (m, 15 H, CO$(CH_2)_4CH_3$, 2 × CH(CH_3)$_2$); ^{13}C NMR ($CDCl_3$) δ 177.70, 172.98, 83.12, 65.31, 64.53, 50.02, 40.40, 33.92, 32.36, 32.03, 31.14, 29.25, 29.06, 25.28, 24.42, 22.16, 21.39, 21.26, 19.27, 19.08, 13.78; FABMS (m/z, relative intensity) 371 (MH^+, 62), 99 ($C_5H_{11}CO^+$, 99). Anal. ($C_{21}H_{38}O_5$) C, H.

(*Z*)-{1-(Hydroxymethyl)-4-[4-methyl-3-(methylethyl)-pent-2-enylidene]-3-oxo-2-oxolanyl}methyl Acetate (7). Standard monoacylation conditions (method A) starting from **52** (0.201 g, 0.8 mmol) were applied. After chromatography with different gradients of hexanes:EtOAc (2:1) and (1:1) the monoacetate product **7** (0.166 g, 70%) was isolated, together with 0.051 g (18%) of the diacetate. Compound **7**: oil; IR ($CHCl_3$) 3595 (OH), 3469 (OH), 3024−2966, 1744 (C═O), 1629 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.27 (d, 1 H, J = 12.1 Hz, >C═CHC*H*═C(i-Pr)$_2$), 7.05 (dt, 1 H, J = 12.2, 2.1 Hz, >C═C*H*CH═C(i-Pr)$_2$), 4.23 (AB d, 1 H, J = 11.9 Hz, C*H*HOAc), 4.17 (AB d, 1H, J = 11.9 Hz, CH*H*OAc), 3.68 (AB d, 1 H, J = 12.2 Hz, C*H*HOH), 3.62 (AB d, 1 H, J = 12.2 Hz, CH*H*OH), 3.08 (heptuplet, 1 H, J = 6.9 Hz, *H*CMe$_2$), 2.92 (dd, 1 H, J = 17.2, 2.2 Hz, H-5_a), 2.75 (dd, 1 H, J = 17.2, 2.0 Hz, H-5_b), 2.45 (heptuplet, 1 H, J = 6.8 Hz, *H*CMe$_2$), 2.25 (br s, 1H, OH), 2.05 (s, 3H, COCH_3), 1.06 (m, 12 H, HC(CH_3)$_2$); ^{13}C NMR ($CDCl_3$) δ 170.88, 168.89, 166.31, 135.82, 119.06, 116.82, 82.17, 65.25, 64.61, 33.56, 29.65, 29.51, 24.10, 24.07, 21.18, 20.66; FABMS (m/z, relative intensity) 311 (MH^+, 100). Anal. ($C_{17}H_{26}O_5$) C, H.

(*E*)-{1-(Hydroxymethyl)-4-[4-methyl-3-(methylethyl)-pent-2-enylidene]-3-oxo-2-oxolanyl}methyl Acetate (8). Standard monoacylation conditions (method A) starting from **53** (0.202 g, 0.75 mmol) were applied. After chromatography with different gradients of hexanes:EtOAc (2:1), (1:1), and (1:2), the monoacetate product **8** (0.133 g, 56%) was isolated, together with 0.079 g (30%) of the diacetate. Compound **8**: solid; mp 61−62 °C; IR ($CHCl_3$) 3446 (OH), 2964−2830, 1748 (C═O), 1636 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.51 (dm, 1 H, J = 12.2 Hz, >C═C*H*CH═C(i-Pr)$_2$), 5.86 (d, 1 H, J = 12.2 Hz, >C═CHC*H*═Ci-Pr$_2$), 4.25 (AB d, 1 H, J = 11.9 Hz, C*H*HOAc), 4.19 (AB d, 1H, J = 11.9 Hz, CH*H*OAc), 3.71 (AB d, 1H, J = 12.1 Hz, C*H*HOH), 3.65 (AB d, 1 H, J = 12.1 Hz, CH*H*OH), 3.15 (heptuplet, 1 H, J = 6.9 Hz, C*H*Me$_2$), 2.90 (dd, 1 H, J = 17.6, 2.8 Hz, H-5_a), 2.73 (dd, 1 H, J = 17.6, 2.7 Hz, H-5_b), 2.49 (heptuplet, 1 H, J = 6.8 Hz, *H*CMe$_2$), 2.30 (br s, 1 H, OH), 2.06 (s, 3 H, COCH_3), 1.05 (d, 12 H, J = 6.9 Hz, HC(CH_3)$_2$); ^{13}C NMR ($CDCl_3$) δ ppm: 171.20, 170.87, 169.05, 132.76, 121.64, 117.29, 82.98, 65.43, 64.76, 30.53, 30.13, 29.75, 24.08, 24.06, 21.08, 20.66; FABMS (m/z, relative intensity) 311 (MH^+, 100). Anal. ($C_{17}H_{26}O_5$) C, H.

(*Z*)-{1-(Hydroxymethyl)-4-[4-methyl-3-(methylethyl)-pent-2-enylidene]-3-oxo-2-oxolanyl}methyl Butanoate (9). Standard monoacylation conditions (method A) starting from **52** (0.203 g, 0.8 mmol) were applied. After chromatography with different gradients of hexanes:EtOAc (3:1), (2:1), and (1:1), the monoacylated product **9** (0.161 g, 63%) was isolated together with 0.016 g (21%) of the diacylated material. Compound **9**: oil, IR ($CHCl_3$) 3458 (OH), 2964−2872, 1746 (C═O), 1632 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.28 (d, 1 H, J = 12.1 Hz, >C═CHC*H*═C(i-Pr)$_2$), 7.04 (dm, 1 H, J = 12.2, >C═C*H*CH═C(i-Pr)$_2$), 4.25 (AB d, 1 H, J = 11.9 Hz, C*H*HOC(O)n-Pr), 4.17 (AB d, 1H, J = 11.9 Hz, CH*H*OC(O)n-Pr), 3.68 (AB d, 1 H, J = 12.2 Hz, C*H*HOH), 3.61 (AB d, 1 H, J = 12.2 Hz, CH*H*OH), 3.09 (heptuplet, 1 H, J = 6.9 Hz, *H*CMe$_2$), 2.93 (dd, 1 H, J = 17.2, 2.1 Hz, H-5_a), 2.75 (dd, 1 H, J = 17.2, 1.9 Hz, H-5_b), 2.46 (heptuplet, 1 H, J = 6.8 Hz, *H*CMe$_2$), 2.28 (t, 2 H, J = 7.4 Hz, COCH_2CH$_2$CH$_3$), 2.19 (br s, 1 H, OH), 1.60 (sextuplet, 2 H, COCH$_2$CH_2CH$_3$), 1.06 (m, 12 H, HC(CH_3)$_2$), 0.91 (t, 3 H, J = 7.4 Hz, COCH$_2$CH$_2$CH_3); ^{13}C NMR ($CDCl_3$) δ 173.48, 168.88, 166.24, 135.70, 119.17, 116.83, 82.25, 65.14, 64.65, 35.93, 33.62, 29.65, 29.50, 24.12, 24.07, 21.18, 18.27, 13.61; FABMS (m/z, relative intensity) 339 (MH^+, 100), 269 (MH−CH_3CH_2-CH═CO^+, 33). Anal. ($C_{19}H_{30}O_5$) C, H.

(*E*)-{1-(Hydroxymethyl)-4-[4-methyl-3-(methylethyl)-pent-2-enylidene]-3-oxo-2-oxolanyl}methyl Butanoate (10). Standard monoacylation conditions (method A) starting from **53** (0.202 g, 0.8 mmol) were applied. After chromatography with hexanes:EtOAc (2:1) the monoacylated product **10** (0.153 g, 80%) was isolated together with 0.071 g (20%) of the diacylated material. Compound **10**: mp 71−72 °C; IR ($CHCl_3$) 3596 (OH), 3470 (OH), 3026−2967, 1742 (C═O), 1636 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.50 (dm, 1 H, J = 12.2 Hz, >C═C*H*CH═C(i-Pr)$_2$), 5.85 (d, 1 H, J = 12.2 Hz, >C═CHC*H*═Ci-Pr$_2$), 4.25 (AB d, 1 H, J = 11.9 Hz, C*H*HOC(O)n-Pr), 4.21 (AB d, 1H, J = 11.9 Hz, CH*H*OC(O)n-Pr), 3.70 (AB d, 1H, J = 12.2 Hz, C*H*HOH), 3.64 (AB d, 1 H, J = 12.2 Hz, CH*H*OH), 3.14 (heptuplet, 1 H, J = 6.9 Hz, C*H*Me$_2$), 2.90 (dd, 1 H, J = 17.6, 2.8 Hz, H-5_a), 2.72 (dd, 1 H, J = 17.6, 2.6 Hz, H-5_b), 2.48 (heptuplet, 1 H, J = 6.9 Hz, *H*CMe$_2$), 2.42 (br s, 1 H, OH), 2.28 (t, 2 H, J = 7.4 Hz, COCH_2CH$_2$CH$_3$), 1.59 (sextuplet, 2 H, J = 7.4 Hz, COCH$_2$CH_2CH$_3$) 1.05 (d, 12 H, J = 6.9 Hz, HC(CH_3)$_2$), 0.89 (t, 3 H, J = 7.4 Hz, COCH$_2$CH$_2$CH_3); ^{13}C NMR ($CDCl_3$) δ 173.50, 171.26, 168.93, 132.59, 121.81, 117.31, 83.16, 65.33, 64.77, 35.89, 30.52, 30.12, 29.74, 24.08, 24.04, 21.09, 21.05, 18.26, 13.60; FABMS (m/z, relative intensity) 339 (MH^+, 100), 269 (MH−CH_3CH_2CH═CO^+, 33). Anal. ($C_{19}H_{30}O_5$) C, H.

(*Z*)-{1-(Hydroxymethyl)-4-[4-methyl-3-(methylethyl)-pent-2-enylidene]-3-oxo-2-oxolanyl}methyl Hexanoate (11). Standard monoacylation conditions (method A) starting from **52** (0.205 g, 0.8 mmol) were applied. After chromatography with different gradients of hexanes:EtOAc (4:1), (2:1), and (1:1), the monoacylated product **11** (0.185 g, 67%) was isolated together with 0.069 g (25%) of the diacylated material. Compound **11**: oil; IR (neat) 3446 (OH), 2961−2871, 1744 (C═O), 1631 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.28 (d, 1 H, J = 12.1 Hz, >C═CHC*H*═C(i-Pr)$_2$), 7.04 (dm, 1 H, J = 12.1, >C═C*H*CH═C(i-Pr)$_2$), 4.25 (AB d, 1 H, J = 11.8 Hz, C*H*HOC(O)$(CH_2)_4CH_3$), 4.16 (AB d, 1H, J = 11.8 Hz, CH*H*OC(O)$(CH_2)_4CH_3$), 3.68 (AB d, 1 H, J = 12.2 Hz, C*H*HOH), 3.61 (AB d, 1 H, J = 12.2 Hz, CH*H*OH), 3.08 (heptuplet, 1 H, J = 6.9 Hz, *H*CMe$_2$), 2.93 (dd, 1 H, J = 17.2, 2.1 Hz, H-5_a), 2.75 (dd, 1 H, J = 17.2, 1.9 Hz, H-5_b), 2.46 (heptuplet, 1 H, J = 6.8 Hz, *H*CMe$_2$), 2.30 (t, 2 H, J = 7.4 Hz, COC$H_2(CH_2)_3CH_3$), 2.26 (br s, 1 H, OH), 1.57 (quintet, 2 H, J = 7.5 Hz, COCH$_2$C$H_2(CH_2)_2CH_3$), 1.25 (m, 4 H, OCO$(CH_2)_2(CH_2)_2CH_3$), 1.06 (m, 12 H, HC(CH_3)$_2$), 0.85 (t, 3 H, J = 6.8 Hz, OCO$(CH_2)_4CH_3$); ^{13}C NMR ($CDCl_3$) δ 173.67, 169.01, 166.12, 135.65, 119.28, 116.83, 82.37, 65.19, 64.63, 33.53, 34.00, 31.19, 29.64, 29.48, 24.45, 24.07, 21.17, 22.21, 13.83; FABMS (m/z, relative intensity) 367 (MH^+, 100), 269 (MH−$CH_3(CH_2)_3CH$═CO^+, 30). Anal. ($C_{21}H_{34}O_5$) C, H.

(*E*)-{1-(Hydroxymethyl)-4-[4-methyl-3-(methylethyl)-pent-2-enylidene]-3-oxo-2-oxolanyl}methyl Hexanoate (12). Standard monoacylation conditions (method A) starting from **53** (0.208 g, 0.8 mmol) were applied. After chromatog-

raphy with different gradients of hexanes:EtOAc (3:1), (2:1), and (1:1), the monoacylated product **12** (0.178 g, 62%) was isolated together with 0.066 g (18%) of the diacylated material. Compound **12**: oil, IR (neat): 3447 (O−H), 2961−2871, 1744 (C=O), 1637 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.51 (dm, 1 H, J = 12.2 Hz, >C=C*H*CH=C(*i*-Pr)$_2$), 5.86 (d, 1 H, J = 12.2 Hz, >C=CHC*H*=C*i*-Pr$_2$), 4.26 (AB d, 1 H, J = 11.9 Hz, C*H*HOC(O)-$(CH_2)_4CH_3$), 4.19 (AB d, 1H, J = 11.9 Hz, CH*H*OC(O)$(CH_2)_4$-CH_3), 3.70 (AB d, 1H, J = 12.2 Hz, C*H*HOH), 3.67 (AB d, 1 H, J = 12.2 Hz, CH*H*OH), 3.15 (heptuplet, 1 H, J = 6.9 Hz, C*H*Me$_2$), 2.90 (dd, 1 H, J = 17.6, 2.7 Hz, H-5$_a$), 2.72 (dd, 1 H, J = 17.6, 2.7 Hz, H-5$_b$), 2.49 (heptuplet, 1 H, J = 6.9 Hz, *H*CMe$_2$), 2.30 (t, 2 H, J = 7.4 Hz, $COCH_2(CH_2)_3CH_3$), 2.12 (br s, 1 H, OH), 1.55 (quintuplet, 2 H, J = 7.4 Hz, $COCH_2CH_2$-$(CH_2)_2CH_3$), 1.26 (m, 4 H, $CO(CH_2)_2CH_2CH_2CH_3$), 1.06 (d, 12 H, J = 6.9 Hz, $HC(CH_3)_2$), 0.85 (t, 3 H, J = 7.4 Hz, CO-$(CH_2)_4CH_3$); ^{13}C NMR ($CDCl_3$) δ 173.70, 171.17, 168.98, 132.66, 121.72, 117.29, 83.08, 65.29, 64.80, 33.99, 31.18, 30.53, 30.14, 29.74, 24.45, 24.08, 24.06, 21.07, 21.05, 22.21, 13.83; FABMS (*m*/*z*, relative intensity) 367 (MH^+, 100), 269 ($MH-CH_3(CH_2)_3$-$CH=CO^+$, 71). Anal. ($C_{21}H_{34}O_5$) C, H.

(*Z*)-{1-(Hydroxymethyl)-4-[4-methyl-3-(methylethyl)-pentylidene]-3-oxo-2-oxolanyl}methyl Acetate (13). Standard monoacylation conditions (method B) starting from **54** (0.102 g, 0.4 mmol) were applied. After chromatography with different gradients of hexanes:EtOAc (4:1), (3:1), (2:1), and (1:1), the monoacetate product **13** (0.078 g, 66%) was isolated together with 0.034 g (25%) of the diacetate. Compound **13**: oil; IR (neat) 3451 (OH), 2958−2873, 1749 (C=O), 1667 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.34 (m, 1 H, >C=C*H*CH_2−CH(*i*-Pr)$_2$), 4.32 (AB d, 1 H, J = 11.9 Hz, C*H*HOAc), 4.24 (AB d, 1 H, J = 11.9 Hz, CH*H*OAc), 3.77 (AB d, 1 H, J = 12.0 Hz, C*H*HOH), 3.71 (AB d, 1 H, J = 12.0 Hz, CH*H*OH), 2.95 (m, 1 H, H-5$_a$), 2.80 (m, 3 H, H-5$_b$, >C=CHCH_2−CH(*i*-Pr)$_2$), 2.30 (br s, 1 H, OH), 2.16 (s, 3 H, $COCH_3$), 1.85 (sextuplet, 2 H, C*H*Me$_2$), 1.19 (m, 1 H, C*H*(*i*-Pr)$_2$), 0.94 (d, 6 H, J = 6.8 Hz, $HC(CH_3)_2$), 0.98 (d, 6H, J = 6.8 Hz, $HC(CH_3)_2$); ^{13}C NMR ($CDCl_3$) δ 170.64, 168.68, 147.57, 122.56, 82.18, 65.34, 64.48, 51.08, 33.08, 29.29, 29.24, 26.26, 21.57, 21.55, 20,59, 19.39, 19.33; FABMS (*m*/*z*, relative intensity) 313 (MH^+, 100), 271 (MH^+-CH_2CO, 36). Anal. ($C_{17}H_{28}O_5$) C, H.

(*E*)-{1-(Hydroxymethyl)-4-[4-methyl-3-(methylethyl)-pentylidene]-3-oxo-2-oxolanyl}methyl Acetate (14). Standard monoacylation conditions (method A) starting from **55** (0.232 g, 0.9 mmol) were applied. After chromatography with different gradients of hexanes:EtOAc (4:1), (3:1), (2:1), and (1:1), the monoacetate product **14** (0.166 g, 61%) was isolated together with 0.060 g (19%) of the diacetate. Compound **14**: oil; IR (neat) 3452 (OH), 2958−2874, 1749 (C=O), 1676 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.84 (tt, 1 H, J = 7.3, 2.7 Hz, >C=C*H*CH_2−CH(*i*-Pr)$_2$), 4.35 (AB d, 1 H, J = 11.8 Hz, C*H*HOAc), 4.25 (AB d, 1 H, J = 11.8 Hz, CH*H*OAc), 3.80 (AB d, 1 H, J = 11.8 Hz, C*H*HOH), 3.72 (AB d, 1 H, J = 11.8 Hz, CH*H*OH), 2.91 (dm, 1 H, J = 17.2 Hz, H-5$_a$), 2.75 (d, 1 H, J = 17.2 Hz, H-5$_b$), 2.66 (m, 1 H, OH), 2.20 (m, 2 H, >C=CHCH_2−CH(*i*-Pr)$_2$), 2.14 (s, 3 H, $COCH_3$), 1.86 (sextuplet, 2 H, C*H*Me$_2$), 1.29 (m, 1 H, C*H*(*i*-Pr)$_2$), 0.98 (d, 6 H, J = 6.6 Hz, $HC(CH_3)_2$), 0.92 (d, 6H, J = 6.8 Hz, $HC(CH_3)_2$); ^{13}C NMR ($CDCl_3$) δ 170.09, 169.53, 143.21, 124.29, 82.55, 65.04, 64.07, 49.73, 29.33, 28.68, 28.60, 28.14, 21.08, 20.99, 18.81, 18.69, 20.06; FABMS (*m*/*z*, relative intensity) 313 (MH^+, 100). Anal. ($C_{17}H_{28}O_5$) C, H.

(*Z*)-{1-(Hydroxymethyl)-4-[4-methyl-3-(methylethyl)-pentylidene]-3-oxo-2-oxolanyl}methyl Butanoate (15). Standard monoacylation conditions (method A) starting from **54** (0.100 g, 0.4 mmol) were applied. After chromatography with different gradients of hexanes:EtOAc (4:1), (3:1), (2:1), and (1:1), the monoacylated product **15** (0.077 g, 61%) was isolated together with 0.031 g (20%) of the diacylated material. Compound **15**: oil; IR (neat) 3446 (OH), 2959−2875, 1744 (C=O), 1670 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.34 (m, 1 H, >C=C*H*CH_2-CH(*i*-Pr)$_2$), 4.35 (AB d, 1H, J = 11.8 Hz, C*H*HOCO*n*-Pr), 4.23 (AB d, 1 H, J = 11.8 Hz, CH*H*OCO*n*-Pr), 3.76 (AB d, 1 H, J = 12.1, C*H*HOH), 3.70 (AB d, 1 H, J = 12.1 Hz, CH*H*OH), 2.95 (m, 1 H, H-5$_a$), 2.76 (m, 1 H, H-5$_b$, >C=CHCH_2CH(*i*-Pr)$_2$), 2.39 (t, 2 H, J = 7.5 Hz, $COCH_2CH_2CH_3$), 2.20 (m, 2 H, >C=CHCH_2CH(*i*-Pr)$_2$), 1.84 (heptuplet, 2 H, C*H*Me$_2$), 1.72 (sextuplet, 2 H, $COCH_2CH_2CH_3$), 1.19 (m, 1 H, C*H*(*i*-Pr)$_2$), 1.02 (t, 3 H, J = 7.6 Hz, $COCH_2CH_2CH_3$), 0.94 and 0.98 (2d, 12 H, J = 6.84 Hz, 2 × $HC(CH_3)_2$); ^{13}C NMR ($CDCl_3$) δ 173.26, 168.76, 147.45, 122.67, 82.31, 65.15, 64.50, 51.06, 35.81, 33.05, 29.26, 29.24, 26.23, 21.57, 21.53, 19.37, 19.34, 18.23, 13.55; FABMS (*m*/*z*, relative intensity) 341 (MH^+, 100), 271 ($MH^+-C_3H_6CO$, 44). Anal. ($C_{19}H_{32}O_5$) C, H.

(*E*)-{1-(Hydroxymethyl)-4-[4-methyl-3-(methylethyl)-pentylidene]-3-oxo-2-oxolanyl}methyl Butanoate (16). Standard monoacylation conditions (method A) starting from **55** (0.230 g, 0.9 mmol) were applied. After chromatography with different gradients of hexanes:EtOAc (4:1), (3:1), (2:1), and (1:1), the monoacylated product **16** (0.161 g, 55%) was isolated together with 0.076 g (22%) of the diacylated material. Compound **16**: oil; IR (neat) 3456 (OH), 2960−2875, 1744 (C=O), 1676, 1176 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.88 (m, 1 H, >C=C*H*CH_2CH(*i*-Pr)$_2$), 4.36 (AB d, 1H, J = 11.9 Hz, C*H*HOCO*n*-Pr), 4.24 (AB d, 1 H, J = 11.9 Hz, CH*H*OCO*n*-Pr), 3.80 (AB d, 1 H, J = 12.2, C*H*HOH), 3.72 (AB d, 1 H, J = 12.2 Hz, CH*H*OH), 2.91 (dd, 1 H, J = 19.2, 2.2 Hz, H-5$_a$), 2.74 (dd, 1 H, J = 19.2, 2.2 Hz, H-5$_b$), 2.38 (t, 2 H, J = 7.5 Hz, $COCH_2CH_2$-CH_3), 2.20 (m, 2 H, >C=CHCH_2CH(*i*-Pr)$_2$), 1.86 (heptuplet, 2 H, C*H*Me$_2$), 1.70 (sextuplet, 2 H, $COCH_2CH_2CH_3$), 1.28 (m, 1 H, C*H*(*i*-Pr)$_2$), 1.01 (t, 3 H, J = 7.5 Hz, $COCH_2CH_2CH_3$), 0.98 and 0.92 (2d, 12 H, J = 6.84 Hz, 2 × $HC(CH_3)_2$); ^{13}C NMR ($CDCl_3$) δ 172.68, 169.52, 143.11, 124.38, 82.63, 64.83, 64.12, 49.71, 35.29, 29.32, 28.66, 28.60, 28.12, 21.07, 20.98, 18.73, 18.72, 17.72, 13.05; FABMS (*m*/*z*, relative intensity) 341 (MH^+, 74), 271 ($MH^+-C_3H_6CO$, 67), 71 ($C_3H_7CO^+$, 100). Anal. ($C_{19}H_{32}O_5$) C, H.

(*Z*)-{1-(Hydroxymethyl)-4-[4-methyl-3-(methylethyl)-pentylidene]-3-oxo-2-oxolanyl}methyl Hexanoate (17). Standard monoacylation conditions (method A) starting from **54** (0.232 g, 0.9 mmol) were applied. After chromatography with different gradients of hexanes:EtOAc (4:1), (3:1), (2:1), and (1:1), the monoacylated product **17** (0.183 g, 58%) was isolated together with 0.098 g (24%) of the diacylated material. Compound **17**: oil; IR (neat) 3447 (OH), 2958−2872, 1745 (C=O), 1670 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.37 (m, 1 H, >C=C*H*CH_2-CH(*i*-Pr)$_2$), 4.34 (AB d, 1H, J = 11.9 Hz, C*H*HOCO$(CH_2)_4CH_3$), 4.23 (AB d, 1 H, J = 11.9 Hz, CH*H*OCO$(CH_2)_4CH_3$), 3.71 (m, 2 H, CH_2OH), 2.99 (m, 1 H, H-5$_a$), 2.82 (m, 3 H, H-5$_b$, >C=CHCH_2CH(*i*-Pr)$_2$), 2.41 (t, 2 H, J = 7.5 Hz, $COCH_2(CH_2)_3$-CH_3), 1.84 (m, 2 H, C*H*Me$_2$), 1.69 (m, 2 H, $COCH_2CH_2(CH_2)_2$-CH_3), 1.37 (m, 4 H, $CO(CH_2)_2(CH_2)_2CH_3$), 1.19 (m, 1 H, C*H*(*i*-Pr)$_2$), 0.96 (m, 15 H, $CO(CH_2)_4CH_3$, 2 × $HC(CH_3)_2$); ^{13}C NMR ($CDCl_3$) δ 173.49, 168.52, 147.65, 122.49, 82.12, 65.07, 64.57, 51.10, 33.92, 33.13, 31.16, 29.28, 26.28, 24.44, 22.20, 21.57, 19.37, 13.82; FABMS (*m*/*z*, relative intensity) 369 (MH^+, 100), 271 ($MH^+-C_5H_{10}CO$, 54). Anal. ($C_{21}H_{36}O_5$) C, H.

(*E*)-{1-(Hydroxymethyl)-4-[4-methyl-3-(methylethyl)-pentylidene]-3-oxo-2-oxolanyl}methyl Hexanoate (18). Standard monoacylation conditions (method A) starting from **55** (0.241 g, 0.9 mmol) were applied. After chromatography with different gradients of hexanes:EtOAc (4:1), (3:1), (2:1), and (1:1), the monoacylated product **18** (0.187 g, 57%) was isolated together with 0.092 g (22%) of the diacylated material. Compound **18**: oil; IR (neat) 3447 (OH), 2957−2873, 1744 (C=O), 1676 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.88 (m, 1 H, >C=C*H*CH_2-CH(*i*-Pr)$_2$), 4.36 (AB d, 1H, J = 11.9 Hz, C*H*HOCO$(CH_2)_4CH_3$), 4.24 (AB d, 1 H, J = 11.9 Hz, CH*H*OCO$(CH_2)_4CH_3$), 3.79 (AB d, 1 H, J = 12.1, C*H*HOH), 3.72 (AB d, 1 H, J = 12.1 Hz, CH*H*OH), 2.90 (dd, 1 H, J = 17.1, 2.3 Hz, H-5$_a$), 2.72 (dd, 1 H, J = 19.2, 2.2 Hz, H-5$_b$), 2.70 (br s, 1 H, OH), 2.38 (t, 2 H, J = 7.6 Hz, $COCH_2(CH_2)_3CH_3$), 2.20 (m, 2 H, >C=CHCH_2CH(*i*-Pr)$_2$), 1.86 (m, 2 H, C*H*Me$_2$), 1.67 (m, 2 H, $COCH_2CH_2(CH_2)_2$-CH_3), 1.34 (m, 5 H, $CO(CH_2)_2(CH_2)_2CH_3$, C*H*(*i*-Pr)$_2$), 0.95 (m, 15 H, $CO(CH_2)_4CH_3$, 2 × $HC(CH_3)_2$); ^{13}C NMR ($CDCl_3$) δ 172.88, 169.48, 143.11, 124.36, 82.61, 64.81, 64.13, 49.72, 33.39, 30.63, 28.66, 28.62, 29.33, 28.13, 23.89, 21.67, 21.06, 21.00, 18.79, 18.73, 13.29; FABMS (*m*/*z*, relative intensity) 369 (MH^+, 83), 271 ($MH^+-C_5H_{10}CO$, 66). Anal. ($C_{21}H_{36}O_5$) C, H.

(*Z*)-{1-(Hydroxymethyl)-4-[4-methyl-3-(methylethyl)-pentylidene]-3-oxo-2-oxolanyl}methyl Octanoate (19). Standard monoacylation conditions (method B) starting from **54** (0.115 g, 0.4 mmol) were applied. After chromatography with different gradients of hexanes:EtOAc (9:1), (4:1), and (3:1), the monoacylated product **19** (0.102 g, 60%) was isolated together with 0.026 g (12%) of the diacylated material. Compound **19**: oil, IR (neat) 3446 (OH), 2957−2855, 1744 (C=O), 1676 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.34 (m, 1 H, >C=C*H*CH_2-CH(*i*-Pr)$_2$), 4.34 (AB d, 1H, J = 11.9 Hz, C*H*HOCO$(CH_2)_6CH_3$), 4.23 (AB d, 1 H, J = 11.9 Hz, CH*H*OCO$(CH_2)_6CH_3$), 3.76 (AB d, 1 H, J = 12.0, C*H*HOH), 3.70 (AB d, 1 H, J = 12.0 Hz, CH*H*OH), 2.99 (m, 1 H, H-5_a), 2.77 (m, 3 H, H-5_b, >C=CHCH_2-CH(*i*-Pr)$_2$), 2.41 (t, 2 H, J = 7.5 Hz, COC$H_2(CH_2)_5CH_3$), 1.85 (septuplet, 2 H, C*H*Me$_2$), 1.69 (m, 2 H, $COCH_2CH_2(CH_2)_4CH_3$), 1.35 (m, 8 H, $CO(CH_2)_2(CH_2)_4CH_3$), 1.19 (m, 1 H, C*H*(*i*-Pr)$_2$), 0.94 and 0.99 (m, 15 H, $CO(CH_2)_6CH_3$, 2 × HC(CH_3)$_2$); ^{13}C NMR ($CDCl_3$) δ 173.50, 168.55, 147.63, 122.51, 82.14, 65.08, 64.54, 51.10, 33.96, 33.13, 31.56, 29.28, 28.90, 28.81, 26.27, 24.75, 22.52, 21.57, 19.37, 13.99; FABMS (*m*/*z*, relative intensity) 397 (MH^+, 7), 57 (C_4H_9+, 100). Anal. ($C_{23}H_{40}O_5$) C, H.

(*E*)-{1-(Hydroxymethyl)-4-[4-methyl-3-(methylethyl)-pentylidene]-3-oxo-2-oxolanyl}methyl Octanoate (20). Standard monoacylation conditions (method A) starting from **55** (0.205 g, 0.8 mmol) were applied. After chromatography with different gradients of hexanes:EtOAc (9:1), (4:1), and (3:1), the monoacylated product **20** (0.183 g, 60%) was isolated together with 0.090 g (22%) of the diacylated material. Compound **20**: oil; IR (neat) 3446 (OH), 2957−2872, 1744 (C=O), 1676 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.90 (m, 1 H, >C=C*H*CH_2-CH(*i*-Pr)$_2$), 4.36 (AB d, 1H, J = 11.8 Hz, C*H*HOCO$(CH_2)_6CH_3$), 4.24 (AB d, 1 H, J = 11.8 Hz, CH*H*OCO$(CH_2)_6CH_3$), 3.80 (AB d, 1 H, J = 12.2, C*H*HOH), 3.72 (AB d, 1 H, J = 12.2 Hz, CH*H*OH), 2.90 (dd, 1 H, J = 17.3, 1.9 Hz, H-5_a), 2.75 (dd, 1 H, J = 17.3, 1.9 Hz, H-5_b), 2.80 (br s, 1 H, OH), 2.38 (t, 2 H, J = 7.6 Hz, COC$H_2(CH_2)_5CH_3$), 2.20 (br t, $J \approx$ 6.5 Hz, 2 H, >C=CHCH_2CH(*i*-Pr)$_2$), 1.86 (sextuplet, 2 H, C*H*Me$_2$), 1.67 (m, 2 H, $COCH_2CH_2(CH_2)_4CH_3$), 1.34 (m, 9 H, $CO(CH_2)_2(CH_2)_4CH_3$, C*H*(*i*-Pr)$_2$), 0.92−0.98 (m, 15 H, $CO(CH_2)_6CH_3$, 2 × HC(CH_3)$_2$); ^{13}C NMR ($CDCl_3$) δ 173.42, 170.05, 143.70, 123.83, 83.16, 65.29, 64.64, 50.22, 33.92, 31.53, 29.82, 29.17, 29.12, 28.96, 28.79, 28.65, 24.71, 22.50, 21.55, 21.51, 19.30, 19.25, 13.98; FABMS (*m*/*z*, relative intensity) 397 (MH^+, 26), 271 (MH^+−$C_7H_{14}CO$). Anal. ($C_{23}H_{40}O_5$) C, H.

(*Z*)-{1-(Hydroxymethyl)-4-[4-methyl-3-(methylethyl)-pentylidene]-3-oxo-2-oxolanyl}methyl Decanoate (21). Standard monoacylation conditions (method B) starting from **54** (0.115 g, 0.4 mmol) were applied. After chromatography with different gradients of hexanes:EtOAc (9:1; 4:1; 3:1) the monoacylated product **21** (0.101 g, 56%) was isolated together with 0.029 g (12%) of the diacylated material. Compound **21**: oil; IR (neat) 3440 (OH), 2956−2855, 1745 (C=O), 1666 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.34 (m, 1 H, >C=C*H*CH_2CH(*i*-Pr)$_2$), 4.34 (AB d, 1H, J = 12.0 Hz, C*H*HOCO$(CH_2)_8CH_3$), 4.23 (AB d, 1 H, J = 12.0 Hz, CH*H*OCO$(CH_2)_8CH_3$), 3.76 (AB d, 1 H, J = 12.0, C*H*HOH), 3.70 (AB d, 1 H, J = 12.0 Hz, CH*H*OH), 3.00 (m, 1 H, H-5_a), 2.80 (m, 3 H, H-5_b, >C=CHCH_2CH(*i*-Pr)$_2$), 2.41 (t, 2 H, J = 7.6 Hz, COC$H_2(CH_2)_7CH_3$), 2.33 (br s, 1 H, OH), 1.90 (sextuplet, 2 H, C*H*Me$_2$), 1.69 (m, 2 H, $COCH_2CH_2(CH_2)_6CH_3$), 1.34 (m, 12 H, $CO(CH_2)_2(CH_2)_6CH_3$), 1.19 (sextuplet, 2 H, C*H*(*i*-Pr)$_2$), 0.94−0.99 (m, 15 H, $CO(CH_2)_8CH_3$, 2 × HC(CH_3)$_2$); ^{13}C NMR ($CDCl_3$) δ 173.49, 168.61, 147.55, 122.57, 82.20, 65.10, 64.52, 51.09, 33.96, 33.11, 31.76, 29.33, 29.17, 29.04, 26.27, 24.75, 22.59, 29.27, 21.27, 19.37, 14.03; FABMS (*m*/*z*, relative intensity) 425 (MH^+, 100), 271 (MH^+−$C_9H_{18}CO$, 69). Anal. ($C_{25}H_{44}O_5$) C, H.

(*E*)-{1-(Hydroxymethyl)-4-[4-methyl-3-(methylethyl)-pentylidene]-3-oxo-2-oxolanyl}methyl Decanoate (22). Standard monoacylation conditions (method A) starting from **55** (0.220 g, 0.9 mmol) were applied. After chromatography with different gradients of hexanes:EtOAc (9:1), (4:1), and (3:1), the monoacylated product **22** (0.228 g, 63%) was isolated together with 0.097 g (21%) of the diacylated material. Compound **22**: oil; IR (neat) 3448 (OH), 2956−2927, 1744 (C=O), 1676 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.90 (m, 1 H, >C=C*H*CH_2-CH(*i*-Pr)$_2$), 4.37 (AB d, 1H, J = 11.8 Hz, C*H*HOCO$(CH_2)_8CH_3$), 4.24 (AB d, 1 H, J = 11.8 Hz, CH*H*OCO$(CH_2)_8CH_3$), 3.80 (AB d, 1 H, J = 12.1, C*H*HOH), 3.72 (AB d, 1 H, J = 12.1 Hz, CH*H*OH), 2.90 (dd, 1 H, J = 17.3, 1.9 Hz, H-5_a), 2.74 (dd, 1 H, J = 17.3, 1.9 Hz, H-5_b), 2.80 (br s, 1 H, OH), 2.39 (t, 2 H, J = 7.5 Hz, COC$H_2(CH_2)_7CH_3$), 2.20 (br t, 2 H, >C=CHCH_2CH(*i*-Pr)$_2$), 1.85 (sextuplet, 2 H, C*H*Me$_2$), 1.67 (m, 2 H, $COCH_2CH_2$-$(CH_2)_6CH_3$), 1.29−1.34 (m, 13 H, $CO(CH_2)_2(CH_2)_6CH_3$, C*H*(*i*-Pr)$_2$), 0.93−0.99 (m, 15 H, $CO(CH_2)_8CH_3$, 2 × HC(CH_3)$_2$); ^{13}C NMR ($CDCl_3$) δ 173.43, 169.75, 143.82, 124.65, 82.88, 65.22, 64.70, 50.24, 33.95, 31.77, 29.91, 29.32, 29.16, 29.04, 28.67, 24.74, 22.59, 29.17, 21.57, 21.53, 19.30, 19.25, 14.04; FABMS (*m*/*z*, relative intensity) 425 (MH^+, 45), 271 (MH^+−$C_9H_{18}CO$, 50). Anal. ($C_{25}H_{44}O_5$) C, H.

(*Z*)-[4-Ethylidene-1-(hydroxymethyl)-3-oxo-2-oxolanyl]-methyl 4-Methyl-3-(methylethyl)pentanoate (23). Standard monoacylation conditions (method B) starting from a mixture of **56** and **57** (0.203 g, 1.2 mmol) were applied. After chromatography with different gradients of hexanes:EtOAc (9:1), (3:1), (2:1), (1:1), and (1:5), four fractions were isolated: (1) a mixture of diacylated *E*- and *Z*-isomers (0.087 g, 16%), (2) 0.102 g (28%) of the desired monoacylated *Z*-isomer **23**, (3) a mixture of *E*- and *Z*-monoacylated isomers (0.021 g, 6%), and (4) 0.092 g (25%) of the monoacylated *E*-isomer **24**. Compound **23**: oil; IR (neat) 3447 (OH), 2961−2876, 1743 (C=O), 1673 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.40 (qt, 1H, J = 7.3, ca. 2.2 Hz, >C=C*H*CH_3), 4.32 (AB d, 1 H, J = 11.8 Hz, C*H*HOCOCH_2CH(*i*-Pr)$_2$), 4.23 (AB d, 1 H, J = 11.8 Hz, CH*H*OCOCH_2CH(*i*-Pr)$_2$), 3.78 (AB d, 1 H, J = 12.2 Hz, C*H*HOH), 3.70 (AB d, 1 H, J = 12.2 Hz, CH*H*OH), 2.97 (dt, 1 H, J = 16.5, ca. 2.2 Hz, H-5_a), 2.79 (dt, 1 H, J = 16.5, ca. 2.2 Hz, H-5_b), 2.61 (br s, 1 H, OH), 2.27 (m, 5 H, >C=CHCH_3, COCH_2CH(*i*-Pr)$_2$), 1.80 (sextuplet, 2 H, C*H*Me$_2$), 1.64 (quintuplet, 1 H, *H*C(*i*-Pr)$_2$), 0.97 and 0.88 (2 d, 12 H, J = 6.8 Hz, 2 × HC(CH_3)$_2$); ^{13}C NMR ($CDCl_3$) δ 174.53, 168.66, 139.73, 124.67, 82.32, 65.24, 64.58, 46.80, 32.95, 32.75, 29.31, 21.27, 18.66, 14.07; FABMS (*m*/*z*, relative intensity) 313 (MH^+, 74). Anal. ($C_{17}H_{28}O_5$) C, H.

(*E*)-[4-Ethylidene-1-(hydroxymethyl)-3-oxo-2-oxolanyl]-methyl 4-Methyl-3-(methylethyl)pentanoate (24). Standard monoacylation conditions (method B) starting from **57** (0.202 g, 1.2 mmol) were applied. After chromatography with different gradients of hexanes:EtOAc (9:1), (3:1), (1:1), and (1:5), the monoacylated product **24** (0.217 g, 58%) was isolated together with 0.070 g (13%) of the diacylated material. Compound **24**: oil; IR (neat) 3450 (OH), 2960, 1743 (C=O), 1683 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.89 (m, 1H, >C=C*H*CH_3), 4.33 (AB d, 1 H, J = 11.8 Hz, C*H*HOCOCH_2CH(*i*-Pr)$_2$), 4.25 (AB d, 1 H, J = 11.8 Hz, CH*H*OCOCH_2CH(*i*-Pr)$_2$), 3.81 (AB d, 1 H, J = 12.2 Hz, C*H*HOH), 3.73 (AB d, 1 H, J = 12.2 Hz, CH*H*OH), 2.91 (dm, 1 H, J = 17.2 Hz, H-5_a), 2.75 (dm, 1 H, J = 17.2 Hz, H-5_b), 2.53 (br s, 1 H, OH), 2.27 (d, 2 H, J = 5.6 Hz, COCH_2-CH(*i*-Pr)$_2$), 1.93 (dt, 3 H, J = 7.3, 2.0 Hz, >C=CHCH_3), 1.80 (sextuplet, 2 H, C*H*Me$_2$), 1.64 (quintuplet, 1 H, *H*C(*i*-Pr)$_2$), 0.96 and 0.87 (2 d, 12 H, J = 6.8 Hz, 2 × HC(CH_3)$_2$); ^{13}C NMR ($CDCl_3$) δ 174.43, 169.90, 136.22, 127.23, 83.33, 65.51, 64.58, 46.79, 32.69, 29.45, 29.26, 29.22, 21.22, 18.59, 15.65; FABMS (*m*/*z*, relative intensity) 313 (MH^+, 100). Anal. ($C_{17}H_{28}O_5$) C, H.

(*Z*)-[4-Butylidene-1-(hydroxymethyl)-3-oxo-2-oxolanyl]-methyl 4-Methyl-3-(methylethyl)pentanoate (25). Standard monoacylation conditions (method B) starting from **58** (0.10 g, 0.5 mmol) were applied. After chromatography with different gradients of hexanes:EtOAc (9:1), (3:1), (1:1), and (1:5), the monoacylated product **25** (0.109 g, 61%) was isolated together with 0.029 g (12%) of the diacylated material. Compound **25**: oil; IR (neat) 3458 (OH), 2960−2874, 1742 (C=O), 1670 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.32 (m, 1H, >C=C*H*$(CH_2)_2$-CH_3), 4.33 (AB d, 1 H, J = 12.0 Hz, C*H*HOCOCH_2CH(*i*-Pr)$_2$), 4.23 (AB d, 1 H, J = 12.0 Hz, CH*H*OCOCH_2CH(*i*-Pr)$_2$), 3.78 (AB d, 1 H, J = 12.2 Hz, C*H*HOH), 3.71 (AB d, 1 H, J = 12.2 Hz, CH*H*OH), 2.96 (m, 1 H, H-5_a), 2.70−2.90 (m, 3 H, H-5_b, >C=CHC$H_2CH_2CH_3$), 2.28 (d, 2 H, COCH_2CH(*i*-Pr)$_2$), 1.81 (sextuplet, 2 H, C*H*Me$_2$), 1.66 (m, 1 H, *H*C(*i*-Pr)$_2$), 1.55

(sextuplet, 2 H, $J \approx 7.3$ Hz, >C=CHCH$_2$CH_2CH$_3$), 1.03 (t, 3 H, $J = 7.3$ Hz, >C=CH(CH$_2$)$_2$CH_3), 0.97 and 0.88 (2 d, 12 H, $J = 6.8$ Hz, 2 × HC(CH_3)$_2$); ^{13}C NMR ($CDCl_3$) δ 174.51, 168.65, 145.02, 123.99, 82.38, 65.31, 64.57, 46.78, 32.92, 32.73, 29.52 (*C*H$_2$), 29.29, 22.22, 21.25, 18.63, 13.66; FABMS (*m*/*z*, relative intensity) 341 (MH$^+$, 89). Anal. ($C_{19}H_{32}O_5$) C, H.

(*E*)-[4-Butylidene-1-(hydroxymethyl)-3-oxo-2-oxolanyl]-methyl 4-Methyl-3-(methylethyl)pentanoate (26). Standard monoacylation conditions (method B) starting from **59** (0.20 g, 1.0 mmol) were applied. After chromatography with different gradients of hexanes:EtOAc (9:1), (3:1), (1:1), and (1:5), the monoacylated product **26** (0.220 g, 64%) was isolated together with 0.061 g (13%) of the diacylated material. Compound **26**: oil; IR (neat) 3450 (OH), 2960−2874, 1742 (C=O), 1680 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.88 (m, 1H, >C=C*H*(CH$_2$)$_2$-CH$_3$), 4.34 (AB d, 1 H, $J = 12.0$ Hz, C*H*HOCOCH$_2$CH(*i*-Pr)$_2$), 4.23 (AB d, 1 H, $J = 12.0$ Hz, CH*H*OCOCH$_2$CH(*i*-Pr)$_2$), 3.81 (AB d, 1 H, $J = 12.2$ Hz, C*H*HOH), 3.73 (AB d, 1 H, $J = 12.2$ Hz, CH*H*OH), 2.91 (dm, 1 H, $J = 17.1$ Hz, H-5$_a$), 2.74 (dm, 1 H, $J = 17.1$ Hz, H-5$_b$), 2.55 (br s, 1 H, OH), 2.27 (d, 2 H, $J = 5.6$ Hz, COCH_2CH(*i*-Pr)$_2$), 2.21 (m, 2 H, >C=CHCH_2CH$_2$CH$_3$), 1.80 (sextuplet, 2 H, C*H*Me$_2$), 1.60 (m, 3 H, *H*C(*i*-Pr)$_2$, >C=CHCH$_2$CH_2CH$_3$), 1.03 (t, 3 H, $J = 7.3$ Hz, >C=CH(CH$_2$)$_2$CH_3), 0.97 and 0.88 (2 d, 12 H, $J = 6.7$ Hz, 2 × HC(CH_3)$_2$); ^{13}C NMR ($CDCl_3$) δ 174.51, 169.75, 141.57, 125.97, 82.99, 65.39, 64.72, 46.81, 32.71, 32.16, 29.74, 29.29, 21.34, 21.27, 18.64, 13.76; FABMS (*m*/*z*, relative intensity) 341 (MH$^+$, 92). Anal. ($C_{19}H_{32}O_5$) C, H.

(*Z*)-[4-Hexylidene-1-(hydroxymethyl)-3-oxo-2-oxolanyl]-methyl 4-Methyl-3-(methylethyl)pentanoate (27). Standard monoacylation conditions (method B) starting from **60** (0.120 g, 0.9 mmol) were employed. After chromatography with different gradients of hexanes:EtOAc (3:1) and (2:1), the monoacylated product **27** (0.134 g, 41%) was isolated together with 0.064 g (15%) of the diacylated material. Compound **27**: oil; IR (neat) 3447 (OH), 2959−2873, 1742 (C=O), 1670 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.33 (tt, 1H, $J = 7.6$, 2.0 Hz, >C=C*H*(CH$_2$)$_4$-CH$_3$), 4.33 (AB d, 1 H, $J = 11.8$ Hz, C*H*HOCOCH$_2$CH(*i*-Pr)$_2$), 4.23 (AB d, 1 H, $J = 11.8$ Hz, CH*H*OCOCH$_2$CH(*i*-Pr)$_2$), 3.78 (AB d, 1 H, $J = 12.2$ Hz, C*H*HOH), 3.71 (AB d, 1 H, $J = 12.2$ Hz, CH*H*OH), 3.01 (dm, 1 H, $J = 16.8$ Hz, H-5$_a$), 2.95 (m, 3 H, H-5$_b$, >C=CHCH_2(CH$_2$)$_3$CH$_3$), 2.28 (d, 2 H, $J = 5.9$ Hz, COCH_2CH(*i*-Pr)$_2$), 1.82 (sextuplet, 2 H, C*H*Me$_2$), 1.67 (m, 1 H, *H*C(*i*-Pr)$_2$), 1.40−1.51 (m, 6 H, >C=CHCH$_2$(CH_2)$_3$CH$_3$), 0.98 (m, 9 H, >C=CH(CH$_2$)$_4$CH_3, HC(CH_3)$_2$), 0.89 (d, $J = 6.8$ Hz, HC(CH_3)$_2$); ^{13}C NMR ($CDCl_3$) δ 174.54, 168.62, 145.39, 123.70, 82.35, 65.29, 64.55, 46.79, 32.93, 32.73, 31.35, 29.31, 29.29, 28.67, 27.65, 27.62, 22.38, 21.27, 18.65, 13.91; FABMS (*m*/*z*, relative intensity) 369 (MH$^+$, 88), 229 (MH$^+$−C$_8$H$_{16}$CO, 35). Anal. ($C_{21}H_{36}O_5$) C, H.

(*E*)-[4-Hexylidene-1-(hydroxymethyl)-3-oxo-2-oxolanyl]-methyl 4-Methyl-3-(methylethyl)pentanoate (28). Standard monoacylation conditions (method B) starting from **61** (0.12 g, 0.9 mmol) were employed. After chromatography with different gradients of hexanes:EtOAc (3:1) and (2:1), the monoacylated product **28** (0.128 g, 40%) was isolated together with 0.086 g (19%) of the diacylated material. Compound **28**: oil; IR (neat) 3447 (OH), 2958−2873, 1742 (C=O), 1679 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.86 (m, 1H, >C=C*H*(CH$_2$)$_4$CH$_3$), 4.35 (AB d, 1 H, $J = 12.0$ Hz, C*H*HOCOCH$_2$CH(*i*-Pr)$_2$), 4.24 (AB d, 1 H, $J = 12.0$ Hz, CH*H*OCOCH$_2$CH(*i*-Pr)$_2$), 3.81 (AB d, 1 H, $J = 12.1$ Hz, C*H*HOH), 3.73 (AB d, 1 H, $J = 12.1$ Hz, CH*H*OH), 2.90 (dm, 1 H, $J = 17.3$ Hz, H-5$_a$), 2.74 (dm, 1 H, $J = 17.3$ Hz, H-5$_b$), 2.28 (d, 2 H, $J = 5.6$ Hz, COCH_2CH(*i*-Pr)$_2$), 2.24 (m, 2 H, >C=CHCH_2(CH$_2$)$_3$CH$_3$), 1.80 (sextuplet, 2 H, C*H*Me$_2$), 1.66 (m, 1 H, *H*C(*i*-Pr)$_2$), 1.39−1.57 (m, 6 H, >C=CHCH$_2$(CH_2)$_3$-CH$_3$), 0.98 (m, 3 H, >C=CH(CH$_2$)$_4$CH_3), 0.97 and 0.88 (2 d, 12 H, $J = 6.8$ Hz, 2 × HC(CH_3)$_2$); ^{13}C NMR ($CDCl_3$) δ 174.53, 169.69, 141.97, 125.64, 82.90, 65.36, 64.74, 46.83, 32.71, 31.40, 30.19, 29.74, 29.32, 29.28, 27.66, 22.35, 21.28, 21.26, 18.66, 18.63, 13.87; FABMS (*m*/*z*, relative intensity) 369 (MH$^+$, 100), 229 (MH$^+$−C$_8$H$_{16}$CO, 65). Anal. ($C_{21}H_{36}O_5$) C, H.

(*Z*)-[4-Decylidene-1-(hydroxymethyl)-3-oxo-2-oxolanyl]-methyl 4-Methyl-3-(methylethyl)pentanoate (29). Standard monoacylation conditions (method B) starting from **62** (0.26 g, 0.9 mmol) were employed. After chromatography with different gradients of hexanes:EtOAc (9:1), 8:2), (3:1), and (1:1), the monoacylated product **29** (0.199 g, 51%) was isolated together with 0.031 g (6.5%) of the diacylated material. Compound **29**: oil; IR (neat) 3450 (OH), 2925−2857, 1744 (C=O), 1671 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.32 (br t, 1H, $J = 7.6$ Hz, >C=C*H*(CH$_2$)$_8$CH$_3$), 4.33 (AB d, 1 H, $J = 12.0$ Hz, C*H*HOCOCH$_2$CH(*i*-Pr)$_2$), 4.23 (AB d, 1 H, $J = 12.0$ Hz, CH*H*OCOCH$_2$CH(*i*-Pr)$_2$), 3.78 (AB d, 1 H, $J = 12.1$ Hz, C*H*HOH), 3.71 (AB d, 1 H, $J = 12.1$ Hz, CH*H*OH), 3.01 (dm, 1 H, $J = 15.0$ Hz, H-5$_a$), 2.85 (m, 3 H, H-5$_b$, >C=CHCH_2(CH$_2$)$_7$CH$_3$), 2.28 (d, 2 H, $J = 5.6$ Hz, COCH_2CH(*i*-Pr)$_2$), 1.82 (sextuplet, 2 H, 2 × C*H*Me$_2$), 1.66 (m, 1 H, *H*C(*i*-Pr)$_2$), 1.34−1.51 (m, 14 H, >C=CHCH$_2$(CH_2)$_7$CH$_3$), 0.88−0.98 (m, 15 H, >C=CH(CH$_2$)$_8$CH_3, 2 × HC(CH_3)$_2$); ^{13}C NMR ($CDCl_3$) δ 174.51, 168.58, 145.36, 123.69, 82.33, 65.30, 64.55, 46.79, 32.94, 32.73, 31.79, 29.42, 29.31, 29.21, 29.10, 29.03, 27.68, 22.59, 21.27, 18.65, 14.03; FABMS (*m*/*z*, relative intensity) 425 (MH$^+$, 38). Anal. ($C_{25}H_{44}O_5$) C, H.

(*E*)-[4-Decylidene-1-(hydroxymethyl)-3-oxo-2-oxolanyl]-methyl 4-Methyl-3-(methylethyl)pentanoate (30). Standard monoacylation conditions (method B) starting from **63** (0.26 g, 0.9 mmol) were employed. After chromatography with different gradients of hexanes:EtOAc (9:1), (8:2), (3:1), and (1:1), the monoacylated product **30** (0.193 g, 50%) was isolated together with 0.035 g (6.5%) of the diacylated material. Compound **30**: oil; IR (neat) 3449 (OH), 2956−2855, 1742 (C=O), 1679 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.85 (m, 1H, >C=C*H*(CH$_2$)$_8$-CH$_3$), 4.35 (AB d, 1 H, $J = 11.9$ Hz, C*H*HOCOCH$_2$CH(*i*-Pr)$_2$), 4.23 (AB d, 1 H, $J = 11.9$ Hz, CH*H*OCOCH$_2$CH(*i*-Pr)$_2$), 3.80 (AB d, 1 H, $J = 12.2$ Hz, C*H*HOH), 3.73 (AB d, 1 H, $J = 12.2$ Hz, CH*H*OH), 2.90 (AB d, 1 H, $J = 17.0$ Hz, H-5$_a$), 2.73 (AB d, 1 H, $J = 17.0$ Hz, H-5$_b$), 2.28 (d, 2 H, $J = 5.9$ Hz, COCH_2CH-(*i*-Pr)$_2$), 2.23 (m, 2 H, >C=CHCH_2(CH$_2$)$_7$CH$_3$), 1.82 (sextuplet, 2 H, 2 × C*H*Me$_2$), 1.65 (m, 1 H, *H*C(*i*-Pr)$_2$), 1.34−1.55 (m, 14 H, >C=CHCH$_2$(CH_2)$_7$CH$_3$), 0.98 (m, 3 H, >C=CH(CH$_2$)$_8$CH_3), 0.97 and 0.88 (2 d, 12 H, $J = 6.6$ Hz, 2 × HC(CH_3)$_2$); ^{13}C NMR ($CDCl_3$) δ 174.47, 169.98, 141.71, 125.88, 83.17, 65.47, 64.65, 46.82, 32.70, 31.76, 30.21, 29.64, 29.31, 29.27, 29.19, 28.00, 22.57, 21.24, 18.64, 14.01; FABMS (*m*/*z*, relative intensity) 425 (MH$^+$, 83). Anal. ($C_{25}H_{44}O_5$), C, H.

(*Z*)-{1-(Hydroxymethyl)-4-[4-methyl-3-(methylethyl)-pentylidene]-3-oxo-2-oxolanyl}methyl 4-Methyl-3-(methylethyl)pentanoate (31). Standard monoacylation conditions (method B) starting from **54** (0.100 g, 0.4 mmol) were employed. After chromatography with different gradients of hexanes:EtOAc (4:1) and (3:1), the monoacylated product **31** (0.065 g, 43%) was isolated as an oil; IR (neat) 3448 (OH), 2959−2874, 1744 (C=O), 1666 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.34 (m, 1 H, >C=C*H*CH$_2$CH(*i*-Pr)$_2$), 4.33 (AB d, 1H, $J = 11.8$ Hz, C*H*HOCOCH$_2$CH(*i*-Pr)$_2$), 4.21 (AB d, 1 H, $J = 12.0$ Hz, CH*H*OCOCH$_2$CH(*i*-Pr)$_2$), 3.73 (m, 2 H, CH_2OH), 2.99 (dm, 1 H, $J = 14.4$ Hz, H-5$_a$), 2.80 (m, 3 H, H-5$_b$, >C=CHCH_2CH(*i*-Pr)$_2$), 2.35 (m, 1 H, OH), 2.28 (d, 2 H, $J = 5.6$ Hz, COCH_2CH-(*i*-Pr)$_2$) 1.84 (heptuplet, 4 H, 4 × C*H*Me$_2$), 1.68 (m, 1 H, COCH$_2$C*H*(*i*-Pr)$_2$), 1.19 (m, 1 H, >C=CHCH$_2$C*H*(*i*-Pr)$_2$), 0.89−0.99 (m, 24 H, 4 × HC(CH_3)$_2$); ^{13}C NMR ($CDCl_3$) δ 174.58, 168.50, 147.67, 122.51, 82.14, 65.22, 64.63, 50.11, 46.79, 33.14, 32.70, 29.31, 29.27, 26.29, 21.61, 21.57, 21.29, 19.39, 19.36, 18.67, 18.65; FABMS (*m*/*z*, relative intensity) 411 (MH$^+$, 23). Anal. ($C_{24}H_{42}O_5$) C, H.

(*E*)-{1-(Hydroxymethyl)-4-[4-methyl-3-(methylethyl)-pentylidene]-3-oxo-2-oxolanyl}methyl 4-Methyl-3-(methylethyl)pentanoate (32). Standard monoacylation conditions (method B) starting from **55** (0.200 g, 0.7 mmol) were employed. After chromatography with different gradients of hexanes:EtOAc (4:1) and (3:1), the monoacylated product **32** (0.120 g, 40%) was isolated as an oil: IR (neat) 3450 (OH), 2958−2874, 1743 (C=O), 1676 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.91 (m, 1 H, >C=C*H*CH$_2$CH(*i*-Pr)$_2$), 4.37 (AB d, 1H, $J = 12.0$ Hz, C*H*HOCOCH$_2$CH(*i*-Pr)$_2$), 4.22 (AB d, 1 H, $J = 12.0$ Hz, CHHOCOCH$_2$CH(*i*-Pr)$_2$), 3.81 (AB d, 1 H, $J = 12.1$, C*H*HOH), 3.73 (AB d, 1 H, $J = 12.1$ Hz, CH*H*OH), 2.91 (dd, 1 H, $J =$

17.5, 2.3 Hz, H-5$_a$), 2.76 (dd, 1 H, J = 17.5, 2.2 Hz, H-5$_b$), 2.29 (d, 2 H, J = 5.6 Hz, COCH_2CH(i-Pr)$_2$), 2.21 (dd, 2 H, J = 7.3, 5.9 Hz, >C=CHCH_2CH(i-Pr)$_2$), 1.83 (heptuplet, 4 H, 4 × CHMe$_2$), 1.66 (m, 1 H, COCH$_2$CH(i-Pr)$_2$), 1.30 (m, 1H, >C=CHCH$_2$CH(i-Pr)$_2$), 0.88−1.00 (m, 24 H, 4 × HC(CH_3)$_2$); ^{13}C NMR ($CDCl_3$) δ 174.51, 169.89, 143.75, 124.79, 83.01, 65.43, 64.74, 50.22, 46.79, 32.68, 29.88, 29.30, 29.15, 28.64, 21.55, 21.27, 19.27, 18.66, 18.63; FABMS (m/z, relative intensity) 411 (MH^+, 17). Anal. ($C_{24}H_{42}O_5$·0.2H_2O) C, H.

(1*S*,2*R*,3*S*,5*S*)-3-(Hydroxymethyl)-7,7-dimethyl-4,6,8-trioxabicyclo[3.3.0]octan-2-ol (1,2-*O*-isopropylidene-α-L-xylofuranose) (74). This compound was prepared according to literature procedures.[63,64]

(1*S*,2*R*,3*S*,5*S*)-7,7-Dimethyl-4,6,8-trioxa-3-[(phenylmethoxy)methyl]bicyclo[3.3.0]octan-2-ol (75). A stirred solution of 1,2-*O*-isopropylidene-α-L-xylofuranose (**74**, 20 g, 105 mmol) in toluene (500 mL) was treated with bis(tributyltin) oxide and refluxed for 5 h in a flask equipped with a Dean−Stark apparatus. After the reaction reached room temperature, it was treated with benzyl bromide (15 mL, 126 mmol) and tetrabutylammonium bromide (17 g, 52.5 mmol). The temperature was raised to 90 °C, and stirring was continued for 60 h. The reaction mixture was then allowed to cool and was concentrated. The residue was purified by flash column chromatography on silica gel with EtOAc:hexanes (1:5) and (1:1) as eluant to give **75** as a clear oil (29 g, 98.4%): ^{1}H NMR ($CDCl_3$) δ 7.20−7.40 (m, 5 H, Ph), 5.96 (d, 1 H, J = 3.6 Hz, H-5), 4.63 (d of AB, 1 H, J = 11.9 Hz, PhCHHO), 4.55 (d of AB, 1 H, J = 11.9 Hz, PhCHHO), 4.49 (d, 1 H, J = 3.6 Hz, H-1), 4.20−4.30 (m, 2 H, H-3, H-2), 3.92 (m, 2 H, PhCH_2OCH_2), 3.60 (br s, 1 H, OH), 1.46 (s, 3 H, CH_3), 1.30 (s, 3 H, CH_3). This compound was used without further purification in the next step.

(1*R*,3*S*,5*S*)-7,7-Dimethyl-4,6,8-trioxa-3-[(phenylmethoxy)methyl)bicyclo[3.3.0]octan-2-one (76). A solution of **75** (14 g, 50 mmol) and acetic acid (5.2 mL, 90 mmol) in CH_2Cl_2 (100 mL) was added dropwise to a stirred suspension of pyridinium dichromate (56.43 g, 150 mmol) and 4 Å molecular sieve (30 g) in CH_2Cl_2 (100 mL). *Note:* due to the strong exothermic nature of this reaction the addition must be done very slowly and with great care. After the additon was finished, stirring continued for 1 h. The reaction mixture was then treated with Celite and ether (400 mL) and stirred for 20 min. Following filtration through a short pad of silica gel and further elution with ether and EtOAc, the combined filtrate was concentrated. The residue was purified by flash column chromatography on silica gel with EtOAc:hexanes (1:2) as eluant to give **76** as an oil (13.62 g, 98%): $[\alpha]_D$ = 147.30 (c 4.25, $CHCl_3$); IR (neat) 1774 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.20−7.40 (m, 5 H, Ph), 6.12 (d, 1 H, J = 4.5 Hz, H-5), 4.40−4.55 (m, 3 H, H-4, PhCH_2O), 4.33 (d, 1 H, J = 4.5 Hz, H-1), 3.70 (d of AB, 2 H, J = 2.4 Hz, PhCH$_2$OCH_2), 1.43 (s, 3 H, CH_3), 1.40 (s, 3 H, CH_3); ^{13}C NMR ($CDCl_3$) δ 209.94 (s), 137.39 (s), 128.47 (d), 127.85 (d), 127.51 (d), 114.11 (s), 103.57 (d), 79.91 (d), 76.81 (d), 73.69 (t), 70.08 (t), 27.61 (q), 27.17 (q). Anal. ($C_{15}H_{18}O_5$) C, H.

(1*S*,2*S*,3*S*,5*S*)-2-(3-Hydroxypropyl)-7,7-dimethyl-4,6,8-trioxa-3[(phenylmethoxy)methyl)bicyclo[3.3.0]octan-2-ol (77). A solution of 3-chloropropanol (2.84 g, 30 mmol) in THF (10 mL) was cooled to −20 °C and treated dropwise while stirring with a solution of methylmagnesium chloride (10 mL, 3 M in THF). The reaction mixture was allowed to warm to room temperature, and magnesium (1.10 g, 45 mmol) was added. The resulting suspension was refluxed and treated at 0, 1, and 2 h intervals with dibromoethane (0.02 mL). After 3 h, the solution was cooled to room temperature. A solution of **76** (2.78 g, 10 mmol) in THF (10 mL) was added dropwise to the above Grignard reagent, and after stirring for 1 h the reaction was cooled over ice and quenched by the addition of a saturated solution of NH_4Cl (20 mL). The reaction mixture was filtered, and the filtrate was extracted several times with EtOAc. The combined organic extract was dried ($MgSO_4$) and concentrated. The residue was purified by flash column chromatography on silica gel with EtOAc:hexanes (4:1) as eluant to give **77** (2.84 g, 85%) as white solid: mp 103 °C; $[\alpha]_D$ −3.340 (c 2.4, $CHCl_3$); IR (neat) 3450 and 3281 (OH) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.20−7.40 (m, 5 H, Ph), 5.78 (d, 1 H, J = 3.9 Hz, H-5), 4.62 (d of AB, 1 H, J = 12.2 Hz, PhCHHO), 4.52 (d of AB, 1 H, J = 12.2 Hz, PhCHHO), 4.29 (d, 1 H, J = 3.9 Hz, H-1), 4.01 (dd, 1 H, J = 3.3, 7.4 Hz, H-3), 3.50−3.72 (m, 4 H, PhCH$_2$OCH_2 and CH_2OH), 1.40−1.80 (m, 4 H, CH_2CH_2CH$_2$-OH), 1.56 (s, 3 H, CH_3), 1.34 (s, 3 H, CH_3); ^{13}C NMR ($CDCl_3$) δ 137.88 (s), 128.41 (d), 127.83 (d), 127.71 (d), 112.45 (s), 103.82 (d), 81.77 (d), 80.43 (d), 78.77 (s), 73.54 (t), 67.98 (t), 62.80 (t), 27.34 (t), 26.75 (q), 26.54 (q), 25.24 (t). Anal. ($C_{18}H_{26}O_6$) C, H.

(1*S*,5*S*,7*S*,8*S*)-3,3-Dimethyl-2,4,6-trioxa-7-[(phenylmethoxy)methyl]spiro[bicyclo[3.3.0]octane-8,5′-oxolane]-10-one (73). A solution of **77** (2.84 g, 8.4 mmol) in CH_2Cl_2 (100 mL) was treated with pyridinium chlorochromate (7.24 g, 33.6 mmol) and 4 Å molecular sieve (8.4 g). After being stirred for 1 h at room temperature, the reaction mixture was quenched with ether and Celite. It was stirred further for 30 min more and filtered through a pad of silica gel. The filtrate and EtOAc washings were collected and reduced to dryness before purification by silica gel with EtOAc:hexanes (2:1) as eluant to give **73** (2.75 g, 98%) as a colorless oil: $[\alpha]_D$ −44.50 (c 1.50, $CHCl_3$); IR (neat) 1785 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.20−7.40 (m, 5 H, Ph), 5.80 (d, 1 H, J = 3.7 Hz, H-5), 4.53 (s, 2 H, PhCH_2O), 4.45 (t, 1 H, J = 5.0 Hz, H-7), 4.37 (d, 1 H, J = 3.7 Hz, H-1), 3.65 (m, 2 H, PhCH$_2$OCH_2), 2.55 (m, 2 H, H-11$_{a,b}$), 2.40 (m, 1 H, H-12$_a$), 1.91 (m, 1 H, H-12$_b$), 1.59 (s, 3 H, CH_3), 1.34 (s, 3 H, CH_3); ^{13}C NMR ($CDCl_3$) δ 175.12 (s), 137.28 (s), 128.51 (d), 127.95 (d), 127.85 (d), 114.32 (s), 103.54 (d), 87.94 (s), 84.12 (d), 77.37 (d), 73.84 (t), 67.40 (t), 27.90 (t), 26.75 (q), 26.65 (q), 25.83 (t). Anal. ($C_{18}H_{22}O_6$) C, H.

(5*R*)-5-(Hydroxymethyl)-5-[(1*S*)-1-hydroxy-2-(phenylmethoxy)ethyl]oxolan-2-one (78). A solution of **73** (8.36 g, 25 mmol) in THF (150 mL) and H_2O (150 mL) was treated with Dowex H^+ resin (15 g, prewashed with MeOH) and refluxed for 24 h. The reaction mixture was filtered and concentrated in vacuo. The residue was dissolved in EtOAc and treated with $NaHCO_3$ and $MgSO_4$. The suspension was filtered, and the filtrate was concentrated in vacuo to give the intermediate hemiacetal which was used for the next step without further purification. The resulting compound was dissolved in MeOH (150 mL) and H_2O (75 mL) and treated with sodium periodate (10.7 g, 50 mmol). After being stirred for 1 h at room temperature, the reaction mixture was filtered and concentrated in vacuo. The residue was dissolved in EtOAc, dried ($MgSO_4$), and concentrated in vacuo to give the intermediate aldehyde which was reduced immediately without further purificaiton. After it was dissolved in MeOH (200 mL) and cooled to 0 °C, sodium borohydride was added portionwise until the starting material was consumed according to TLC. The reaction mixture was first quenched with acetone and then 20 min after with acetic acid before concentrating in vacuo. The residue was diluted with EtOAc and filtered, and the filtrate was concentrated in vacuo. Purification by flash column chromatography on silica gel with a gradient of EtOAc:hexanes (3:1) to EtOAc:MeOH (9:1) as eluant afforded **78** (5.0 g, 75% in 3 steps) as a syrup: $[\alpha]_D$ −8.000 (c 1.25, $CHCl_3$); IR (neat) 3418 (OH), 1760 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.20−7.40 (m, 5 H, Ph), 4.52 (s, 2 H, PhCH_2O), 3.93 (dd, 1 H, J = 4.1, 6.7 Hz, CH(OH)CH$_2$OCH$_2$-Ph), 3.75 (d of AB, 1 H, J = 12.1 Hz, CH(OH)CH$_2$OCHHPh), 3.61 (d of AB, 1 H, J = 12.1 Hz, CH(OH)CH$_2$OCHHPh), 3.50−3.66 (m, 2 H, CH_2OH), 2.88 (br s, 2 H, OH), 2.50−2.63 (m, 2 H, H-3), 2.08−2.35 (m, 2 H, H-4); ^{13}C NMR ($CDCl_3$) δ 177.73, 137. 25, 128.47, 127.94, 127.77, 88.64, 73.61, 72.37, 69.78, 64.84, 29.00, 25.08. Anal. ($C_{14}H_{18}O_5$) C, H.

(5*R*)-5-[(1*S*)-1,2-Dihydroxyethyl]-5-(hydroxymethyl)oxolan-2-one (79). A solution of **78** (3.0 g, 11.3 mmol) in MeOH (100 mL) was treated with acetic acid (0.1 mL) and hydrogenated for 5 h under a balloon of hydrogen in the presence of 10% palladium on carbon (3.0 g). The reaction mixture was filtered, and the filtrate was concentrated in vacuo. The residue was purified by flash column chromatography on silica gel with $CHCl_3$:MeOH (5:1) as eluant to give the triol as a syrup which crystallized after cooling in the refrigerator to give

79 as a white solid (1.965 g, 99%): mp 78−79 °C; $[\alpha]_D$ −4.20 (*c* 3.68, MeOH); IR (KBr) 3385 (OH), 1758 (C=O) cm^{-1}; ^1H NMR (CD_3OD) δ 3.48−3.84 (m, 5 H), 2.54−2.68 (m, 2 H), 2.10−2.40 (m, 2 H); ^{13}C NMR (CD_3OD) δ 180.3, 91.3, 75.2, 65.3, 63.0, 30.2, 26.1. Anal. ($C_7H_{12}O_5$) C, H.

(5*R*)-5-[(1*S*)-3,3-Dimethyl(2,4-dioxolanyl)]-5-(hydroxymethyl)oxolan-2-one (80). A solution of **79** (1.94 g, 11 mol) in anhydrous acetone (120 mL) was treated with *p*-toluenesulfonic acid (0.13 g, 0.68 mmol) and stirred for 4 h at room temperature. The reaction mixture was neutralized with solid $NaHCO_3$, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel with EtOAc:hexanes (3:1) as eluant to give **80** as a white solid (2.31 g, 97%): mp 69−71 °C; $[\alpha]_D$ −30.840 (*c* 0.83, $CHCl_3$); IR (KBr) 3445 (OH), 1769 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.16 (t, 1 H, $J_{1',5'a} = J_{1',5'b} = 6.8$ Hz, OC*H*CH_2O), 4.06 (dd, 1 H, $J_{5'a,5'b} = 8.5$ Hz, $J_{5'a,1'} = 6.8$ Hz, OCHC*H*HO), 3.93 (dd, 1 H, $J_{5'b,5'a} = 8.5$ Hz, $J_{5'b,1'} = 6.8$ Hz, OCHCH*H*O), 3.67 (d of AB, 1 H, $J = 12.4$ Hz, C*H*HOH), 3.61 (d of AB, 1 H, $J = 12.4$ Hz, CH*H*OH), 2.48−2.80 (m, 2 H, H-3), 2.05−2.35 (m, 2 H, H-4), 1.36 (s, 3 H, CH_3), 1.34 (s, 3 H, CH_3); ^{13}C NMR ($CDCl_3$) δ 178.01, 108.89, 87.34, 78.35, 65.11, 64.73, 29.06, 25.55, 25.50, 25.03. Anal. ($C_{10}H_{16}O_5$) C, H.

(5*R*)-5-[(1*S*)-3,3-Dimethyl(2,4-dioxolanyl)]-5-[(phenylmethoxy)methyl]oxolan-2-one (81). A stirred solution of **80** (2.16 g, 10 mmol) in DMF (20 mL) was allowed to react with benzyl bromide (3.60 mL, 30 mmol) in the presence of silver-(I) oxide (3.48 g, 15 mmol) for 4 days at room temperature. The reaction mixture was filtered, and the filter cake was washed with ether. Water was added to the combined filtrate, and the ether layer was separated. The aqueous layer was then extracted with ether three times, and the combined ether extract was washed with H_2O, 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 **81** as an oil (2.573 g, 84%): IR (neat) 1777 (C=O) cm^{-1}; $[\alpha]_D$ −17.090 (*c* 2.13, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.25−7.35 (m, 5 H, Ph), 4.53 (s, 2 H, PhCH_2O), 4.18 (t, 1 H, $J_{1',5'a} = J_{1',5'b} = 6.9$ Hz, OC*H*CH_2O), 4.02 (dd, 1 H, $J_{5'a,5'b} = 8.7$ Hz, $J_{5'a,1'} = 6.9$ Hz, OCHC*H*HO), 3.93 (dd, 1 H, $J_{5'b,5'a} = 8.7$ Hz, $J_{5'b,1'} = 6.9$ Hz, OCHCH*H*O), 3.50 (s, 2 H, $PhCH_2OCH_2$), 2.48−2.75 (m, 2 H, H-3), 2.0−2.38 (m, 2 H, H-4), 1.35 (s, 3 H, CH_3), 1.33 (s, 3 H, CH_3); ^{13}C NMR ($CDCl_3$) δ 177.02, 137.25, 128.37, 127.77, 127.44, 109.18, 85.61, 78.42, 73.58, 73.11, 64.77, 28.81, 26.61, 25.64, 25.29. Anal. ($C_{17}H_{22}O_5$) C, H.

(5*R*)-5-[(1*S*)-3,3-Dimethyl(2,4-dioxolanyl)]-3-[(*Z*)-4-methyl-3-(methylethyl)pentylidene)-5-[(phenylmethoxy)methyl]oxolan-2-one (82) and (5*R*)-5-[(1*S*)-3,3-Dimethyl-(2,4-dioxolanyl)]-3-[(*E*)-4-methyl-3-(methylethyl)pentylidene)-5-[(phenylmethoxy)methyl]oxolan-2-one (83). A stirred solution of **81** (0.613 g, 2.0 mmol) in THF (8 mL) was cooled to −78 °C and treated slowly with lithium bis(trimethylsilyl)amide (1 M in THF, 2.4 mL, 2.4 mmol). After 30 min, a solution of 4-methyl-3-(methylethyl)pentanal (0.370 g, 2.6 mmol) in hexamethyl-phosphoramide (0.42 mL, 2.4 mmol) and THF (1 mL) was added, and stirring was continued for 2 h at −78 °C. The reaction was quenched with a solution of saturated ammonium chloride and diluted with ether. The organic layer was washed with H_2O, 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 the intermediate β-hydroxy lactone (0.79 g, 89%) as an oil. The compound was dissolved in CH_2Cl_2 (20 mL), cooled to 0 °C, and treated with triethylamine (1.0 mL) and methanesulfonyl chloride (0.29 mL) with stirring for 2 h at room temperature. The reaction mixture was cooled to 0 °C, and 1,8-diazabicyclo[5,4,0]undec-7-ene (1.43 mL) was added. After stirring for 1 h at room temperature, the mixture was concentrated in vacuo. The residue was diluted with ether, washed with 1 N HCl and H_2O, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel with EtOAc:hexanes (1:5) as eluant to give **83** (*Z*-isomer, 0.296 g, 35%) and **82** (*E*-isomer: 0.203 g, 24%) as oils.

***Z*-Isomer 83:** $[\alpha]_D$ +2.620 (*c* 1.0, $CHCl_3$); IR (neat) 1760 (C=O), 1678 (C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.25−7.38 (m, 5 H, Ph), 6.18 (m, 1 H, >C=C*H*), 4.55 (s, 2 H, PhCH_2O), 4.23 (t, 1 H, $J_{1',5'a} = J_{1',5'b} = 6.8$ Hz, OC*H*CH_2O), 3.98 (dd, 1 H, $J_{5'a,5'b} = 8.7$ Hz, $J_{5'a,1'} = 6.8$ Hz, OCHC*H*HO), 3.94 (dd, 1 H, $J_{5'b,5'a} = 8.7$ Hz, $J_{5'b,1'} = 6.8$ Hz, OCHCH*H*O), 3.51 (s, 2 H, $PhCH_2OCH_2$), 2.95 (m, 1 H, H-4a), 2.60−2.85 (m, 3 H, H-4b and >C=CH−CH_2), 1.77 (m, 2 H, 2 × C*H*$(CH_3)_2$), 1.37 (s, 3 H, CH_3), 1.33 (s, 3 H, CH_3), 1.10 (m, 1 H, C*H*(*i*-Pr)$_2$), 0.83−0.92 (m, 12 H, CH_3); ^{13}C NMR ($CDCl_3$) δ 169.04, 145.89, 137.44, 128.33, 127.69, 127.43, 123.36, 109.40, 82.08, 77.45, 73.58, 72.88, 64.60, 51.08, 33.29, 29.23, 29.17, 26.03, 25.79, 25.17, 21.60, 21.51, 19.39, 19.24. Anal. ($C_{26}H_{38}O_5$) C, H.

***E*-Isomer 82:** $[\alpha]_D$ −5.160 (*c* 1.0, $CHCl_3$); IR (neat) 1760 (C=O), 1678 (C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.25−7.38 (m, 5 H, phenyl), 6.74 (m, 1 H, >C=C*H*), 4.55 (s, 2 H, PhCH_2O), 4.26 (t, 1 H, $J_{1',5'a} = J_{1',5'b} = 6.8$ Hz, OC*H*CH_2O), 4.02 (dd, 1 H, $J_{5'a,5'b} = 8.7$ Hz, $J_{5'a,1'} = 6.8$ Hz, OCHC*H*HO), 3.92 (dd, 1 H, $J_{5'b,5'a} = 8.7$ Hz, $J_{5'b,1'} = 7.0$ Hz, OCHCH*H*O), 3.54 (s, 2 H, $PhCH_2OCH_2$), 2.92 (m, 1 H, H-4a), 2.68 (m, 1 H, H-4b), 2.11 (m, 2 H, >C=CH−CH_2), 1.78 (m, 2 H, 2 × C*H*$(CH_3)_2$), 1.36 (s, 3 H, CH_3), 1.33 (s, 3 H, CH_3), 1.10 (1 H, C*H*(*i*-Pr)$_2$), 0.82−0.92 (m, 12 H); ^{13}C NMR ($CDCl_3$) δ 170.20, 142.00, 137.35, 128.29, 127.66, 127.40, 125.49, 109.36, 82.06, 77.38, 73.56, 72.83, 64.57, 50.20, 30.07, 29.05, 28.48, 25.73, 25.09, 21.51, 21.48, 19.24, 19.09. Anal. ($C_{26}H_{38}O_5$) C, H.

Hexyl (*E*)-3-{(1*S*)-(Hydroxymethyl)-4-[(*Z*)-4-methyl-3-(methylethyl)pentylidene]-3-oxo-2-oxalanyl}prop-2-enoate (69). A stirred solution of **83** (0.1 g, 0.2 mmol) in ether (10 mL) was treated with periodic acid (0.27 g, 1.2 mmol) for 24 h at room temperature. The reaction mixture was filtered, and the filtrate was concentrated in vacuo. The residue was purified by flash column chromatography over silica gel with EtOAc:hexanes (1:1) as eluant to give the correponding aldehyde (0.08 g, 0.227 mmol, 96%). The compound was dissolved in CH_2Cl_2 (8 mL) and treated with hexyl (triphenylphosphoranylidene)acetate (0.19 g, 0.5 mmol). The solution was stirred for 24 h at room temperature and concentrated in vacuo. The residue was purified by flash column chromatography over silica gel with EtOAc:hexanes (1:9) as eluant to give intermediate **85** (0.09 g, 0.2 mmol, 93%) as an oil: ^1H NMR ($CDCl_3$) δ 7.25−7.38 (m, 5 H, Ph), 6.92 (d, 1 H, $J = 15.6$ Hz, C*H*=C$HCO_2C_6H_{13}$), 6.19 (m, 1 H, >C=C*H*), 6.14 (d, 1 H, $J = 15.6$ Hz, CH=C*H*$CO_2C_6H_{13}$), 4.57 (AB t, $J = 12.9$ Hz, 2 H, PhCH_2O), 4.13 (t, 1 H, $J = 6.6$ Hz, $CO_2CH_2C_5H_{11}$), 3.53 (s, 2 H, $PhCH_2OCH_2$), 3.10 (m, 1 H, H-5a), 2.66−2.76 (m, 3 H, H-5b, >C=CHCH_2), 1.77 (m, 2 H, 2 × C*H*$(CH_3)_2$), 1.63 (m, 2 H, $CO_2CH_2CH_2C_4H_9$), 1.25−1.40 (m, 6 H, $CO_2(CH_2)_2(CH_2)_3CH_3$), 1.08 (1 H, C*H*(*i*-Pr)$_2$), 0.80−0.92 (m, 15 H). A solution of this compound (0.09 g, 0.2 mmol) in CH_2Cl_2 (15 mL) was cooled to −78 °C, treated with boron trichloride (1 M in CH_2Cl_2, 0.85 mL, 0.85 mmol), and stirred for 2 h at −78 °C. The reaction was quenched with saturated $NaHCO_3$ solution and immediately partitioned between ice-cold ether and water. The organic layer was washed with water, 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 **69** (0.042 g, 50%) as an oil: $[\alpha]_D$ +11.910 (*c* 1.0, $CHCl_3$); IR (KBr) 1760, 1716 (C=O), 1660 (C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.86 (d, 1 H, $J = 15.6$ Hz, C*H*=C$HCO_2C_6H_{13}$), 6.26 (m, 1 H, >C=C*H*), 6.14 (d, 1 H, $J = 15.6$ Hz, CH=C*H*$CO_2C_6H_{13}$), 4.14 (t, 1 H, $J = 6.6$ Hz, $CO_2CH_2C_5H_{11}$), 3.76 (dd, 1 H, $J = 12.1, 6.3$ Hz, C*H*HOH), 3.64 (dd, 1 H, $J = 12.2, 6.3$ Hz, CH*H*OH), 3.13 (m, 1 H, H-5a), 2.66−2.76 (m, 3 H, H-5b and >C=CHCH_2), 2.03 (t, 1 H, OH), 1.77 (m, 2 H, 2 × C*H*$(CH_3)_2$), 1.65 (m, 2 H, $CO_2CH_2CH_2C_4H_9$), 1.25−1.40 (m, 6 H, $CO_2(CH_2)_2(CH_2)_3CH_3$), 1.10 (m, 1 H, C*H*(*i*-Pr)$_2$), 0.80−0.92 (m, 15 H); ^{13}C NMR ($CDCl_3$) δ 168.64, 165.86, 148.23, 145.05, 122.24, 122.13, 83.40, 66.39, 65.04, 51.10, 35.74, 31.37, 29.32, 29.26, 28.50, 26.37, 25.53, 22.48, 21.62, 21.53, 19.39, 19.33, 13.98. Anal. ($C_{23}H_{38}O_5$) C, H.

Hexyl (*E*)-3-{(1*S*)-(Hydroxymethyl)-4-[(*E*)-4-methyl-3-(methylethyl)pentylidene]-3-oxo-2-oxalanyl}prop-2-

enoate (70). This compound was prepared from **82** in 44% yield following the same procedure as for the synthesis of **69**.

Compound 84: ^{1}H NMR ($CDCl_3$) δ 7.25−7.38 (m, 5 H, Ph), 6.95 (d, 1 H, J = 15.8 Hz, C*H*=CHCO$_2$C$_6$H$_{13}$), 6.80 (m, 1 H, >C=C*H*), 6.15 (d, 1 H, J = 15.8 Hz, CH=C*H*CO$_2$C$_6$H$_{13}$), 4.57 (dd of AB, J = 11.9 Hz, 2 H, PhC*H*$_2$O), 4.13 (t, 1 H, J = 6.6 Hz, CO$_2$C*H*$_2$C$_5$H$_{11}$), 3.54 (s, 2 H, PhCH$_2$OC*H*$_2$), 3.10 (dm, 1 H, $J \approx$ 19 Hz, H-5a), 2.68 (dm, 1 H, $J \approx$ 19 Hz, H-5b), 2.08 (m, 2 H, >C=CHC*H*$_2$), 1.76 (m, 2 H, 2 × C*H*(CH$_3$)$_2$), 1.65 (m, 2 H, CO$_2$CH$_2$C*H*$_2$C$_4$H$_9$), 1.25−1.40 (m, 6 H, CO$_2$(CH$_2$)$_2$(C*H*$_2$)$_3$CH$_3$), 1.19 (1 H, C*H*(*i*-Pr)$_2$), 0.80−0.92 (m, 15 H).

Compound 70: $[\alpha]_D$ +24.670 (*c* 0.5, $CHCl_3$); IR (KBr) 1760, 1723 (C=O), 1681 (C=C) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.90 (d, 1 H, J = 15.6 Hz, C*H*=CHCO$_2$C$_6$H$_{13}$), 6.82 (m, 1 H, >C=C*H*), 6.14 (d, 1 H, J = 15.6 Hz, CH=C*H*CO$_2$C$_6$H$_{13}$), 4.14 (t, 1 H, J = 6.6 Hz, CO$_2$C*H*$_2$C$_5$H$_{11}$), 3.80 (d, 1 H, J = 12.2 Hz, C*H*HOH), 3.66 (d, 1 H, J = 12.2 Hz, CH*H*OH), 3.09 (dm, 1 H, $J \approx$ 18 Hz, H-5a), 2.72 (dm, 1 H, $J \approx$ 18 Hz, H-5b), 2.12 (m, 2 H, >C=CHC*H*$_2$), 1.78 (m, 2 H, 2 × C*H*(CH$_3$)$_2$), 1.65 (m, 2 H, CO$_2$-CH$_2$C*H*$_2$C$_4$H$_9$), 1.25−1.40 (m, 6 H, CO$_2$(CH$_2$)$_2$(C*H*$_2$)$_3$CH$_3$), 1.10 (1 H, C*H*(*i*-Pr)$_2$), 0.80−0.92 (m, 15 H); ^{13}C NMR ($CDCl_3$) δ 169.66, 165.78, 144.75, 144.64, 124.03, 122.33, 83.71, 66.72, 65.07, 50.30, 32.46, 31.39, 29.17, 28.73, 28.50, 25.53, 22.50, 21.56, 19.30, 13.98. Anal. (C$_{23}$H$_{38}$O$_5$) C, H.

Acknowledgment. This work was supported in part by the Research Institute of Pharmaceutical Science, College of Pharmacy, Seoul National University. The authors thank Dr. James A. Kelley from the LMC, NCI, for mass spectral analysis and interpretation.

References

(1) For paper 15 in the series, see: Benzaria, S.; Bienfait, B.; Nacro, K.; Wang S.; Lewin, N. E.; Beheshti, M.; Blumberg, P. M.; Marquez, V. E. Conformationally Constrained Analogues of Diacylglycerol. 15. The Indispensable Role of the sn-1 and sn-2 Carbonyls in the Binding of DAG-Lactones to Protein Kinase C (PK-C). *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3403−3408.

(2) Quest, A. F. G. Regulation of Protein Kinase C: A Tale of Lipids and Proteins. *Enzyme Protein* **1996**, *49*, 231−261.

(3) Newton, A. C. Protein Kinase C: Structure, Function, and Regulation. *J. Biol. Chem.* **1995**, *270*, 28495−28498.

(4) Sakai, N.; Sasaki, K.; Ikegaki, N.; Shirai, Y.; Ono, Y.; Saito, N. Direct Visualization of the Translocation of the γ-Subspecies of Protein Kinase C in Living Cells Using Fusion Proteins with Green Fluorescent Protein. *J. Cell. Biol.* **1997**, *139*, 1465−1476.

(5) Oancea, E.; Meyer, T. Protein Kinase C as a Molecular Machine for Decoding Calcium and Diacylglycerol Signals. *Cell* **1998**, *95*, 307−318.

(6) Rhee, S. G.; Bae, Y. S. Regulation of Phosphoinositide-Specific Phospholipase C Isozymes. *J. Biol. Chem.* **1997**, *272*, 15045−15048.

(7) Exton, J. H. Phospholipase D: Enzymology, Mechanisms of Regulation, and Function. *Physiol. Rev.* **1997**, *77*, 303−320.

(8) Livneh, E.; Fishman, D. D. Linking Protein Kinase C to Cell-Cycle Control. *Eur. J. Biochem.* **1997**, *248*, 1−9.

(9) Ono, Y.; Fujii, T.; Igarashi, K.; Kuno, T.; Tanaka, C.; Kikkawa, U.; Nishizuka, Y. Phorbol Ester Binding to Protein Kinase-C Requires a Cysteine-Rich Zinc-Finger-Like Sequence. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 4868−4871.

(10) 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.

(11) Kishi, Y.; Rando, R. R. Structural basis of protein kinase C activation by tumor promoters. *Acc. Chem. Res.* **1998**, *31*, 163−172.

(12) Wender, P. A.; Cribbs, C. M.; Koehler, K. F.; Sharkey, N. A.; Herald, C. L.; Kamano, Y.; Pettit, G. R.; Blumberg, P. M. Modeling of the Bryostatins to the Phorbol Ester Pharmacophore on Protein Kinase C. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 7197−7201.

(13) Hecker, E. In *Carcinogenesis, Vol. 2: Mechanisms of Tumor Promotion and Cocarcinogenesis*; Slaga, T. J., Sivak, A. J., Boutwell, R. K., Eds.; Raven Press: New York, 1978; pp 11−49.

(14) The status of bryostatin in 32 clinical trials can be found on the NCI Web page at http://cancernet.nci.nih.gov/prot/patsrch.shtml.

(15) Wender, P. A.; DeBrabander, J.; Harran, P. G.; Hinkle, K. W.; Lippa, B.; Pettit, G. R. Synthesis and Biological Evaluation of Fully Synthetic Bryostatin Analogues. *Tetrahedron Lett.* **1998**, *39*, 8625−8628.

(16) Wender, P. A.; DeBrabander, J.; Harran, P. G.; Jimenez, J. M.; Koehler, M. F. T.; Lippa, B.; Park, C. M.; Shiozaki, M. Synthesis of the First Members of a New Class of Biologically Active Bryostatin Analogues. *J. Am. Chem. Soc.* **1998**, *120*, 4534−4535.

(17) Wender, P. A.; DeBrabander, J.; Harran, P. G.; Jimenez, J. M.; Koehler, M. F. T.; Lippa, B.; Park, C. M.; Siedenbiedel, C.; Pettit, G. R. The Design, Computer Modeling, Solution Structure, and Biological Evaluation of Synthetic Analogues of Bryostatin 1. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 6624−6629.

(18) Endo, Y.; Ohno, M.; Hirano, M.; Itai, A.; Shudo, K. Synthesis, Conformation, and Biological Activity of Teleocidin Mimics, Benzolactams. A Clarification of the Conformational Flexibility Problem in Structure−Activity Studies of Teleocidins. *J. Am. Chem. Soc.* **1996**, *118*, 1841−1855.

(19) Endo, Y.; Hirano, M.; Driedger, P. E.; Stabel, S.; Shudo, K. Novel Conformationally Constrained Analogues of Diacylglycerol. Protein Kinase C Binding Affinity of Simplified Compounds Based on a six-membered Lactam Moiety *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2997−3000.

(20) Endo, Y.; Shimazu, M.; Fukasawa, H.; Driedger, P. E.; Kimura, K.; Tomioka, N.; Itai, A.; Shudo, K. Synthesis, Computer Modeling and Biological Evaluation of Novel Protein Kinase C Agonists Based on a seven-membered Lactam Moiety. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 173−178.

(21) Qiao, L.; Wang, S.; George, C.; Lewin, N. E.; Blumberg, P. M.; Kozikowski, A. P. Structure-Based Design of a New Class of Protein Kinase C Modulators. *J. Am. Chem. Soc.* **1998**, *120*, 6629−6630.

(22) Kong, F.; Kishi, Y.; Perez-Sala, D.; Rando, R. R. The Pharmacophore of Debromoaplysiatoxin Responsible for Protein Kinase-C Activation. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 1973−1976.

(23) Wang, S.; Milne, G. W. A.; Nicklaus, M. C.; Marquez, V. E.; Lee, J.; Blumberg, P. M. Protein Kinase C Modeling of the Binding Site and Prediction of Binding Constants. *J. Med. Chem.* **1994**, *37*, 1326−1338.

(24) Sharma, R. Lee, J.; Wang, S.; Milne, G. W. A.; Lewin, N. E.; Blumberg, P. M.; Marquez, V. E. Conformationally Constrained Analogues of Diacylglycerol 0.10. Ultrapotent Protein Kinase C Ligands Based on a Racemic 5-Disubstituted Tetrahydro-2-Furanone Template. *J. Med. Chem.* **1996**, *39*, 19−28.

(25) Lee, J.; Wang, S.; Milne, G. W. A.; Sharma, R.; Lewin, N. E.; Blumberg, P. M.; Marquez, V. E. Conformationally Constrained Analogues of Diacylglycerol 0.11. Ultrapotent Protein Kinase C Ligands Based on a Chiral 5-Disubstituted Tetrahydro-2-Furanone Template. *J. Med. Chem.* **1996**, *39*, 29−35.

(26) Lee, J.; Sharma, R.; Wang, S.; Milne, G. W. A.; Lewin, N. E.; Szallasi, Blumberg, P. M.; George. C.; Marquez, V. E. Conformationally Constrained Analogues of Diacylglycerol. 12. Ultrapotent Protein Kinase C Ligands Based on a Chiral 4,4-Disubstituted Heptono-1,4-Lactone Template. *J. Med. Chem.* **1996**, *39*, 36−45.

(27) Zhang, 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.

(28) Ichikawa, S.; Hatanaka, H.; Takeuchi, Y.; Ohno, S.; Inagaki, F. Solution Structure of Cysteine-Rich Domain of Protein Kinase C Alpha. *J. Biochem.* **1995**, *117*, 566−574.

(29) Cochet, C.; Gill, G. N.; Meisenhelder, J.; Cooper, J. A.; Hunter, T. C−Kinase Phosphorylates the Epidermal Growth Factor Receptor and Reduces its Epidermal Growth Factor-Stimulated Tyrosine Protein Kinase Activity. *J. Biol. Chem.* **1984**, *259*, 2553−2558.

(30) Grever, M. R.; Schepartz, S. A.; Chabner, B. A. The National Cancer Institute: Cancer Drug Discovery and Development Program. *Semin. Oncol.* **1992**, *19*, 622−638. Additional information on the NCI in vitro screen can be found at the following Web address: http://dtp.nci.nih.gov/.

(31) Krauter, G.; Von der Lieth, 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.

(32) Sugita, K.; Neville, C. F.; Sodeoka, M.; Sasai, H.; Shibasaki, M. Stereocontrolled Syntheses of Phorbol Analogues and Evaluation of their Binding Affinity to PKC. *Tetrahedron Lett.* **1995**, *36*, 1067−1070.

(33) The molecular graphics in this figure was generated with the program VMD, Humphrey, W.; Dalke, W.; Schulten, K. VMD: Visual Molecular Dynamics. *J. Mol. Graphics* **1996**, *14*, 33−38.

(34) Davis, R. J.; Ganong, B. R.; Bell, R. M.; Czech. Structural Requirements for Diacylglycerol to Mimic Tumor-promoting Phorbol Diester Action on the Epidermal Growth Factor Receptor. *J. Biol. Chem.* **1985**, *260*, 5315−5329.

(35) Ganong, B. R.; Loomis, C. R.; Bell, R. M. Specificity and Mechanism of Protein Kinase C Activation by sn-1,2-Diacylglycerols. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 1184−1188.
(36) Xu, R. X.; Pawelczyk, T.; Xia, T.-H.; Brown, S. C. NMR Structure of a Protein Kinase C−Gamma Phorbol-Binding Domain and Study of Protein−Lipid Micelle Interactions. *Biochemistry* **1997**, *36*, 10709−10717.
(37) Denmark, S. E.; Harmata, M. A.; White, K. S. Studies on the Addition of Allyl Oxides to Sulfonylallenes. Preparation of Highly Substituted Allyl Vinyl Ethers for Carbanaionic Claisen Rearrangements. *J. Org. Chem.* **1987**, *52*, 4031−4042.
(38) Lee, J.; Sharma, R.; Marquez, V. E. Synthesis of Chiral Doubly C-4 Branched γ-Lactones. An Efficient Synthesis of 4-C−Hydroxymethyl-2,3-Dideoxy-Hexono-1,4-Lactones. *Carbohydr. Lett.* **1995**, *1*, 285−290.
(39) KOWWIN 1.63, Syracuse Research Corp., http://esc.syrres.com/.
(40) Kazanietz, M. G.; Areces, L. B.; Bahador, A.; Mischak, H.; Goodnight, J.; Mushinski, J. F.; Blumberg, P. M. Characterization of Ligand and Substrate Specificity for the Calcium-Dependent and Calcium-Independent Protein Kinase-C Isozymes. *Mol. Pharmacol.* **1993**, *44*, 298−307.
(41) Morris, G. M.; Goodsell, D. S.; Huey, R.; Olson, A. J. Distributed Automated Docking of Flexible Ligands to Proteins: Parallel Applications of AutoDock 2.4. *J. Comput.-Aid. Mol. Des.* **1996**, *10*, 293−304.
(42) Teng, K.; Marquez, V. E.; Milne, G. W. A.; Barchi, Jr., J. J.; Kazanietz, M. G.; Lewing, N. E.; Blumberg, P. M.; Abushanab, E. Conformationally Constrained Analogues of Diacylglycerol − Interaction of gamma-Lactones with the Phorbol Ester Receptor of Protein Kinase-C. *J. Am. Chem. Soc.* **1992**, *114*, 1059−1070.
(43) Lee, L. S.; Weinstein, I. B. Tumor-Promoting Phorbol Esters Inhibit Binding of Epidermal Growth Factor to Cellular Receptors. *Science* **1978**, *202*, 313−315.
(44) Dr. Edward Sausville, NCI, personal communication.
(45) Boyd, M. R.; Paull, K. D. Some Practical Considerations and Applications of the National Cancer Institute In Vitro Anticancer Drug Discovery Screen. *Drug Dev. Res.* **1995**, *34*, 91−109.
(46) Paull, K. D.; Shoemaker, R. H.; Hodes, L.; Monks, A.; Scudiero, D. A.; Rubinstein, L.; Plowman, J.; Boyd, M. R. Display and Analysis of Patterns of Differential Activity of Drugs Against Human Tumor Cell Lines: Development of Mean Graph and COMPARE Algorithm. *J. Natl. Cancer Inst.* **1989**, *81*, 1088−1092.
(47) Gulakowski, R. J.; McMahon, J. B.; Buckheit, R. W.; Gustafson, K. R.; Boyd, M. R. Antireplicative and Anticytopathic Activities of Prostratin, a Non-Tumor-Promoting Phorbol Ester, Against Human Immunodeficiency Virus (HIV). *Antivir. Res.* **1997**, *33*, 87−97.
(48) The anticancer screen data on prostratin can be found on the NCI web page by submitting a query at http://dtp.nci.nih.gov/docs/dtp search.html.
(49) Caponigro, F.; French, R. C.; Kaye, S. B. Protein kinase C: A Worthwhile Target for Anticancer Drugs? *Anticancer Drug* **1997**, *8*, 26−33.
(50) Hu, H. Recent Discovery and Development of Selective Protein Kinase C Inhibitors. *Drug Discovery Today* **1996**, *1*, 438−447.
(51) Han, Z. T.; Zhu, X. X.; Yang, R. Y.; Sun, J. Z.; Tian, G. F.; Liu, X. J.; Cao, G. S.; Newmark, H. L.; Conney, A. H.; Chang, R. L. Effect of Intravenous Infusions of 12-O-Tetradecanoylphorbol-13-Acetate (TPA) in Patients with Myelocytic Leukemia: Preliminary Studies on Therapeutic Efficacy and Toxicity. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 5357−5361.
(52) Han, T. H.; Tong, Y. K.; He, L. M.; Zhang, Y.; Sun, J. Z.; Tian, Y. W.; Zhang H.; Cui, Y. L.; Newmark, H. L.; Conney, A. H.; Chang, R. L. 12-O-Tetradecanoylphorbol-13-Acetate (TPA)-Induced Increase in Depressed White Blood Cell Counts in Patients Treated with Cytotoxic Cancer Chemotherapeutic Drugs. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 5362−5365.
(53) Szallasi, Z.; Krsmanovic, L.; Blumberg, P. M. Nonpromoting 12-Deoxyphorbol 13-Esters Inhibit Phorbol 12-Myristate 13-Acetate Induced Tumor Promotion in CD-1 Mouse Skin. *Cancer Res.* **1993**, *53*, 2507−2512.
(54) Szallasi, Z.; Denning, M. F.; Smith, C. B.; Dlugosz, A. A.; Yuspa, S. H.; Pettit, G. R.; Blumberg, P. M. Bryostatin 1 Protects Protein Kinase C-Delta from Down-Regulation in Mouse Keratinocytes in Parallel with its Inhibition of Phorbol Ester-Induced Differentiation. *Mol. Pharmacol.* **1994**, *46*, 840−850.
(55) Blumberg, P. M.; Acs, P.; Bhattacharyya, D. K.; Lorenzo, P. S. In *Signal Transduction and Cell Cycle Inhibitors*; Gutkind, J. S., Ed.; Humana Press: Totawa, NJ, in press.
(56) Molecular Simulation Inc. (http://www.msi.com/).
(57) Sadowski, J.; Gasteiger, J.; Klebe, G. Comparison of Automatic 3-Dimensional Model Builders Using 639 X-ray Structures. *J. Chem. Inf. Comput. Sci.* **1994**, *34*, 1000−1008.
(58) Giesen, D. G.; Hawkins, G. D.; Liotard, D. A.; Cramer, C. J. A Universal Model for the Quantum Mechanical Calculation of Free Energies of Solvation in Non-Aqueous Solvents. *Theor. Chem. Acc.* **1997**, *98*, 85−109.
(59) Gasteiger, J.; Marsili, M. Iterative Partial Equalization of Orbital Electronegativity. Rapid Access to Atomic Partial Charges. *Tetrahedron* **1980**, *36*, 3219−3288.
(60) Kazanietz, M. G.; Areces, L. B.; Bahador, A.; Mischak, H.; Goodnight, J.; Mushinski, J. F.; Blumberg, P. M. Characterization of Ligand and Substrate Specificity for the Calcium-Dependent and Calcium-Independent Protein Kinase-C Isozymes. *Mol. Pharmacol.* **1993**, *44*, 298−307.
(61) Bal, D. S.; Childers, W. E.; Pinnick, H. W. Oxidation of α,β-Unsaturated Aldehydes. *Tetrahedron* **1981**, *37*, 2091−2096.
(62) Hillis, L. R.; Ronald, R. C. Total Synthesis of (−)-Grahamimycin. *J. Org. Chem.* **1985**, *50*, 470−473.
(63) Baker, B. R.; Schaub, R. Puromycin. Synthetic Studies. XIII. Synthesis of 6-Dimethylamino-9-(3′-Amino-3′-Deoxy-β-D-Arabinofuranosyl)Purine. *J. Am. Chem. Soc.* **1955**, *77*, 5900−5905.
(64) Lee, J.; Marquez, V. E.; Lewin, N. E.; Blumberg, P. M. Synthesis of Two Rigid Diacylglycerol Analogues Having a 1,7-Dioxaspiro-[4.4]nonane bis-γ-Butyrolactone Skeleton. 4. *Synlett* **1994**, 206−208.

JM9904607