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Conformationally Constrained Analogues of Diacylglycerol (DAG). Effect on Protein Kinase C (PK-C) Binding by the Isosteric Replacement of Sn-1 and Sn-2 Esters in DAG-lactones

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Abstract—In order to determine the importance of the two ester pharmacophores in high affinity, conformationally constrained DAG-lactones (**Lac-1**–**5**) as PK-C ligands, we have independently replaced the sn-1 and sn-2 carbonyl esters in these compounds by ketone (**2**, **10**, **11**), amide (**3**, **25**–**28**), and hydroxyl (**12**, **13**) isosteres. Although the ketone analogue of the sn-1 ester (**2**) exhibited comparable activity to the parent **Lac-1** when taking into account the difference in lipophilicities, the other isosteres were significantly poorer PK-Cα ligands compared to the parent DAG-lactones. This study demonstrates that the ester functionality in DAG-lactone plays an important role in the ligand's capacity to form a strong hydrogen bond with Gly253 at the active site. The discrete K_i analysis from the sn-1 and sn-2 isosteres further confirms that the DAG-lactones bind preferentially to the C1-domain in the sn-2 binding mode, as previously suggested.

Introduction

The Protein Kinase C (PK-C) family of serine/threonine kinases is implicated in growth factor- and G-protein-coupled receptor signaling and plays a key regulatory role in a variety of cellular functions, including cell growth, differentiation, apoptosis, and cell-cycle control.[1–3] As an endogenous ligand, diacylglycerol (DAG) activates both the calcium-dependent classical PKC isoforms α, β, and γ and the novel or calcium-independent PKC isoforms δ, ε, η and θ by binding to the C1 domains of the enzymes and promoting association with the membrane phospholipids.[4] Phorbol esters, which are used as powerful pharmacological tools for studying PK-C function, bind to the same DAG-binding site of PK-Cs in a competitive manner and display binding affinities several orders of magnitude higher than that of DAG.[5,6] Phorbol esters function as potent structural mimetics of DAG because their conformationally rigid scaffold, unlike the flexible glycerol backbone of DAG, is able to specifically direct the hydrophilic pharmacophores (Fig 1).

Over the past several years, we have explored conformationally constrained DAG analogues to identify structurally simple PK-C ligands with affinities similar to those of the phorbol esters. Among these, we have reported that 5-acyloxymethyl-5-hydroxymethyl-tetrahydro-2-furanones, a class of DAG-lactones, display low nanomolar binding affinities for PK-Cα.[7] Extensive molecular modeling studies, extending our insights derived from the crystal structure of the PK-Cδ–phorbol-13-acetate complex,[8] revealed that when these DAG-lactones are docked into the empty receptor, only one of the two non-equivalent carbonyl esters, sn-1 or sn-2 (nomenclature based on the corresponding DAG backbone as shown in Fig. 2), forms hydrogen bonds with Gly253. The two energetically equivalent orientations are defined, respectively, as sn-1 and sn-2 binding modes depending on which carbonyl group is engaged in binding to the protein.[9] Although in either binding mode one of the carbonyls of the DAG lactones

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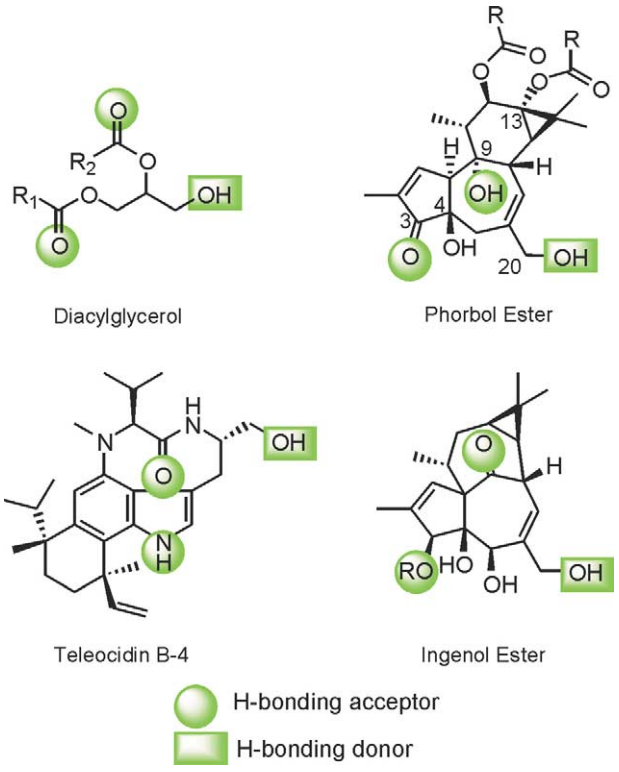


Figure 1.

remained uninvolved in the binary complex with the protein, the removal of either the sn-1 or sn-2 carbonyl in a DAG-lactone caused a dramatic drop in binding affinity, emphasizing the importance of the additional carbonyl group.[10] We hypothesize that the additional carbonyl function, like the C-9(OH)/C-13(C=O) motif in phorbol esters which also appears free of contact with the protein in the phorbol ester/C1b complex, interacts with the phospholipid head groups required for high affinity binding under the conditions of the biological assays.

Although two of the three pharmacophores in DAG-lactones are ester carbonyls, their counterpart pharmacophores in other natural products with affinity for PK-C may contain either a different oxygen type, namely a ketone (phorbol's C_3-C=O and ingenol's C_9-C=O), or an amide (teleocidin's C_{11}-CONH) (Fig. 1). In the present study, we therefore sought to replace both sn-1 and sn-2 carbonyls with ketone, amide and hydroxyl groups to gain additional insight into the importance of these structural changes on a DAG-lactone template for PK-C binding affinity in either the sn-1 or sn-2 binding modalities.

Chemistry

The DAG-lactones (**Lac-1–5**), selected as the parent compounds, were previously reported[11–13] or synthesized by a similar approach. Starting with optically active (*E*)-5-benzyloxymethyl-5-formyl-3-[(*Z*)-9-octadecenyldene]tetrahydro-2-furanone (**1**) reported previously,[12] α,β-unsaturated ketone (**2**) and acetamide (**3**) analogues were synthesized as sn-1 isosteres of DAG-lactone Lac-1 as outlined in Scheme 1.

Syntheses of cyclopentanone and cyclopentanol analogues as sn-2 isosteres are represented in Scheme 2. Alkylation of diethylmalonate with *cis*-1,4-dichloro-2-butene afforded the cyclopentene **4**, whose diester function was reduced and then protected as the acetonide to afford compound **5**. Dihydroxylation of the olefin in **5** and selective monoprotection of a single hydroxyl as a *p*-methoxybenzyl (PMB) ether group was followed by oxidation of the remaining hydroxyl to ketone **7**. Wittig olefination of **7** with tetradecyl triphenylphosphonium bromide and deprotection of PMB followed by oxidation of the corresponding hydroxyl produced an *E*/*Z*-mixture of the α-alkylidene cyclopentanones **8** and **9** in 71% yield with a ratio of 1:1.6. The geometric isomers were readily separated by column chromatography at this stage, and their structural assignments were determined by ^{1}H NMR spectroscopy where the β-vinyl proton of the *E*-isomer (**8**) appears ca. 0.6 ppm downfield from that of the *Z*-isomer (**9**). Each isomer was individually hydrolyzed to remove the isopropylidene group and selectively acylated with pivaloyl chloride to give the racemic cyclopentanone products **10** (*E*-isomer)

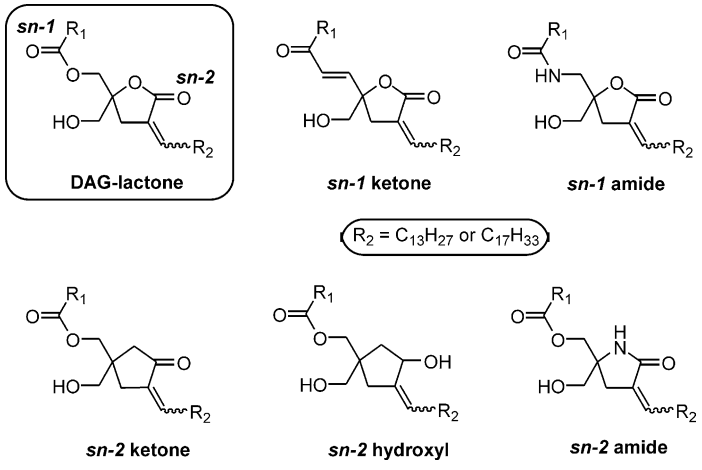
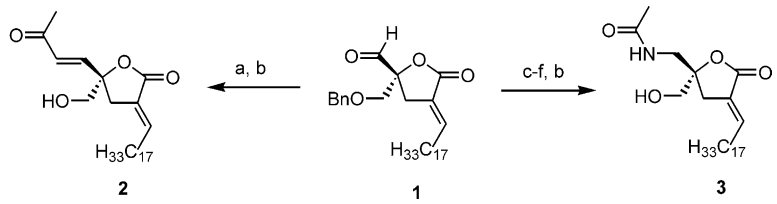
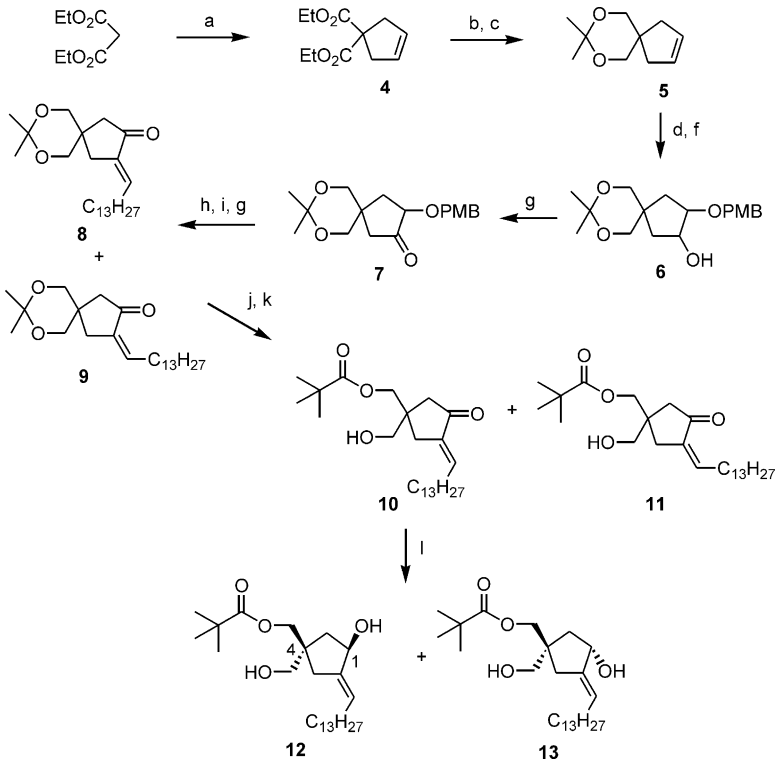


Figure 2.



Scheme 1. Reagents and conditions: (a) $CH_3COCHPPh_3$, CH_2Cl_2, rt; (b) BCl_3, CH_2Cl_2, −78 °C; (c) $NaBH_4$, MeOH, 0 °C; (d) MsCl, NEt_3, CH_2Cl_2; (e) NaN_3, DMF, 100 °C; (f) H_2, Lindler's cat, Ac_2O, EtOAc.



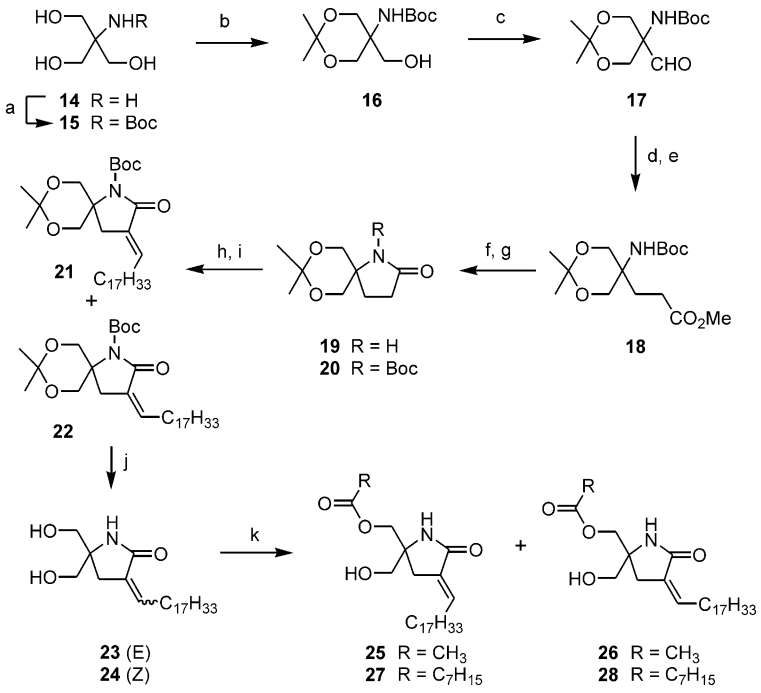
Scheme 2. Reagents and conditions: (a) NaOEt, *cis*-$ClCH_2CH{=}CHCH_2Cl$, EtOH; (b) $LiAlH_4$, Et_2O; (c) $CH_2{=}C(OCH_3)CH_3$, *p*-TsOH, DMF; (d) 4-NMO, OsO_4, acetone; (f) PMBCl, NaH, THF; (g) PCC, 4 Å mol. sieve, CH_2Cl_2; (h) $C_{14}H_{29}PPh_3Br$, *t*-BuOK, THF, benzene; (i) DDQ, CH_2Cl_2–H_2O; (j) p-TsOH, MeOH; (k) $(CH_3)_3CCOCl$, pyridine, CH_2Cl_2; (l) $NaBH_4$, $CeCl_3$–$7H_2O$, CH_2Cl_2–MeOH.

and **11** (*Z*-isomer), respectively. The ensuing selective reduction of (*E*)-cyclopentanone **10** gave a diastereomeric mixture of cyclopentanols **12** and **13**. Assignment of their structures was confirmed by COSY and NOESY experiments in which, for instance, the NOE enhancement between the H-1 and methylene protons of CH_2OH in **12** was examined.

The synthesis of lactam analogues as an additional sn-2 isostere is outlined in Scheme 3. Commercially available trishydroxymethylaminomethane (**14**) was protected with the *t*-BOC group followed by acetonide protection of the diol to give **16**. The remaining primary alcohol of **16** was oxidized to the aldehyde **17**, which was then extended by two carbons to give **18** after Wittig olefination and subsequent hydrogenation of the double bond. Base-catalyzed cyclization of **18** with sodium methoxide formed the lactam ring which occurred concomitantly with removal of the *t*-BOC to give **19**. This compound was reprotected with *t*-BOC to give **20**, and the incorporation of an α-alkylidene chain into lactam **20** proceeded via the aldol product to give the expected *E*/*Z* mixture of **21** and **22**, which were readily separated by column chromatography and were assigned as described for the previous lactone series. Following the same procedure used for the synthesis of cyclopentanone analogues, **21** and **22** were converted to the final target compounds **25**–**28**, respectively.

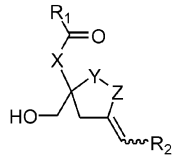
Results and Discussion

The interaction of the sn-1 and sn-2 isosteres of DAG-lactone 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 the recombinant single isozyme, PK-Cα, in the presence of phosphatidylserine as previously described.[9] The inhibition curves obtained for all ligands were of the type expected for competitive inhibition, and the IC_{50} values were determined by fitting the data points to the theoretical noncooperative competitive curve. The K_i values for inhibition of binding were calculated from the corresponding IC_{50} values (Table 1). The parent compounds, **Lac-1**–**5**, and their isosteres



Scheme 3. Reagents and conditions: (a) Boc_2O, NEt_3, MeOH; (b) *p*-TsOH, acetone; (c) PCC, 4 Å mol. sieve, CH_2Cl_2; (d) $PPh_3CHCO_2CH_3$, CH_2Cl_2; (e) H_2, Pd/C, EtOAc; (f) $NaOCH_3$, MeOH; (g) Boc_2O, NEt_3, DMAP, THF; (h) LiHMDS, THF; $C_{17}H_{33}CHO$; (i) MsCl, NEt_3, CH_2Cl_2; DBU (j) CF_3CO_2H, THF–H_2O; (k) CH_3COCl (or $C_7H_{15}COCl$), pyridine, CH_2Cl_2.

Table 1.



	Chirality/Geometry	X	Y	Z	R_1	R_2	cLogP[a]	K_i (nM)
Sn1								
Lac-1	*E* (5*S*)	CH=CH	O	C=O	OCH_3	$C_{17}H_{33}$	7.38	20 (±2.9)[b]
2	*E* (5*S*)	CH=CH	O	C=O	CH_3	$C_{17}H_{33}$	7.06	56.6 (±7.2)
3	*E* (5*S*)	CH_2NH	O	C=O	CH_3	$C_{17}H_{33}$	6.04	1740 (±180)
Sn-2								
Lac-2	*E*	CH_2O	O	C=O	$C(CH_3)_3$	$C_{13}H_{27}$	6.72	7.83 (±0.55)[c]
10	*E*	CH_2O	CH_2	C=O	$C(CH_3)_3$	$C_{13}H_{27}$	7.95	4926 (±85)
12	*E* (1*R**,4*S**)	CH_2O	CH_2	CH–OH	$C(CH_3)_3$	$C_{13}H_{27}$	8.58	3850 (±160)
13	*E* (1*S**,4*R**)	CH_2O	CH_2	CH–OH	$C(CH_3)_3$	$C_{13}H_{27}$	8.58	6480 (±620)
Lac-3	*Z*	CH_2O	O	C=O	$C(CH_3)_3$	$C_{13}H_{27}$	6.72	7.15 (±0.51)[c]
11	*Z*	CH_2O	CH_2	C=O	$C(CH_3)_3$	$C_{13}H_{27}$	7.95	605 (±98)
Lac-4	*E*	CH_2O	O	C=O	CH_3	$C_{17}H_{33}$	7.11	28 (±2.2)[d]
25	*E*	CH_2O	NH	C=O	CH_3	$C_{17}H_{33}$	7.10	5990 (±660)
27	*E*	CH_2O	NH	C=O	C_7H_{15}	$C_{17}H_{33}$	10.04	3789 (±75)
Lac-5	*Z*	CH_2O	O	C=O	CH_3	$C_{17}H_{33}$	7.11	24 (±2.9)[d]
26	*Z*	CH_2O	NH	C=O	CH_3	$C_{17}H_{33}$	7.10	3640 (±140)
28	*Z*	CH_2O	NH	C=O	C_7H_{15}	$C_{17}H_{33}$	10.04	1970 (±100)

[a]calculated according to the fragment-based program KOWWIN (http://esc.syrres.com).
[b]ref 13.
[c]unpublished result.
[d]ref 11.

contain a combination of acetyl or pivaloyl as R_1 on the sn-1 ester and tetradecyl or oleyl as R_2 on the sn-2 ester. The binding affinities of the isosteric ligands were compared with those of the parent compounds with identical R_1 and R_2 groups. As expected, all the parent compounds were identified as high affinity ligands for PKCα, with a range of K_i values of 7.15–28 nM.

Substitution of the sn-1 ester in **Lac-1** ($K_i = 20$ nM) with a ketone caused a 3-fold reduction in binding affinity for compound **2** ($K_i = 56.6$ nM). Considering the relative calculated log P values for **Lac-1** (log P = 7.38) and **2** (log P = 7.06), the intrinsic binding affinities of these compounds are probably very comparable since there is a positive correlation with lipophilicity for this range of

potencies.[14] Substitution of the sn-1 ester in **Lac-1** for an amide resulted in an almost two orders of magnitude loss in the binding affinity of compound **3** ($K_i = 1740$ nM). Although both compounds **2** and **3** contain a carbonyl group capable of effectively hydrogen bonding the NH group of Gly253 based on our previous model,[7] the replacement of the ether oxygen in the sn-1 ester of DAG-lactones with either a carbon or nitrogen atom hampered both derivatives from favorably binding to the enzyme. The result might be explained in two ways. One hypothesis is that the ether oxygen of the ester might play an important role as a hydrogen bonding acceptor for binding with PK-C, because the amide analogue **3**, bearing N–H as the hydrogen bonding donor, was a poorer ligand than the ketone analogue **2**. The alternative is that the electron effect of the ether oxygen on the carbonyl, acting as a hydrogen bonding acceptor in enzyme binding, would be more favorable than those of the carbon and amine groups. However, since both the oxygen and amine are electron-donating atoms and have similiar electronic properties, the latter scenario appears to be less convincing.

In the study of the sn-2 ester isosteres in DAG-lactone, the lactones of **Lac-2**–**5** as the parent compounds were replaced by cyclopentanone **10**, **11**, cyclopentanol **12**, **13**, and lactams **25**–**28**, respectively. However, they displayed even lower binding affinities than those of the sn-1 type. For the geometric *E*-isomer, the affinity of the DAG-lactone with tetradecylidene in R_2 (**Lac-2**, $K_i = 7.83$ nM) dropped 630-fold in cyclopentanone **10** ($K_i = 4926$ nM), and 490- and 830-fold, respectively, in cyclopentanols **12** ($K_i = 3850$ nM) and **13** ($K_i = 6480$ nM). Likewise, the affinity of the DAG-lactone with oleylidene (**Lac-4**, $K_i = 28$ nM) was also reduced 214-fold in the corresponding lactam **25** ($K_i = 5990$ nM) and 135-fold in the lipophilic lactam **27** ($K_i = 3789$ nM). The results suggest that the sn-2 ester of the DAG-lactone is more sensitive to isosteric modification than the sn-1 ester, in terms of binding affinity, and would be regarded as the more crucial pharmacophore as a hydrogen-bonding acceptor.

We reported previously that the replacement of the sn-1 and sn-2 carbonyl esters in DAG-lactones with sulfonate esters as an isostere indicated that their isosteric properties were structurally dependent.[10] While the C=O and SO_2 groups appeared to be true isosteres when forced to reside near the lipid interface by the adjacent hydrophobic alkyl chain, they did not show equivalent isosteric properties when they existed away from the lipid bilayer, at a site where they are expected to bind to the C1 domain of PK-C. However, in contrast to the aspects of the sulfonate ester discussed previously, most of the ester isosteres utilized in these study, including the ketone, hydroxyl, and amide, except for the ketone analogue of the sn-1 ester, did not serve as adequate isosteres regardless of their proximity to the lipid interface. Regarding the lactam template as a PK-C ligand, Kozikowski's group recently reported that 5(*S*)-hydroxymethyl-2,3-disubstituted-pyrrolidone analogues designed as PK-C ligands, which have a similar template as our γ-lactams **25**–**28**, displayed a moderate binding affinity to PK-Cα ($K_i = > 300$ nM depending on Log P).[15] In addition, their molecular modeling study revealed that the N–H of the pyrrolidone skeleton formed an additional hydrogen bond with the C=O of Leu251 in PK-Cδ, which participated in hydrogen bonding with the C_{20}-OH of phorbol in the X-ray structure of PK-Cδ C1b in complex with phorbol 13-acetate. Although they demonstrated that the pyrrolidone template might be another surrogate of teleocidin, a natural PK-C activator, the binding affinities (K_i) of their γ-lactam analogues were much poorer than those of our γ-lactone analogues, which have affinities in the low nM range. Therefore, it might be concluded that the oxygen of the ester has an advantage over the nitrogen of the amide in interacting with the C1-domain of PK-C. Even though the sn-1 and sn-2 isosteric analogues were not profitable as PK-C ligand candidates, the degree of the reduction in affinity provides information regarding the two binding modes of DAG-lactone to the enzyme proposed previously.[9] The reductions in the sn-1 ester isosteres were calculated as ca. 3-fold in **2** (ketone) and 87-fold in **3** (amide) as compared with the parent compound (ester), **Lac-1**. However, those in the sn-2 ester were severely reduced, as represented by the 630-fold loss in **10** (ketone) and the 216-fold loss in **25** (amide) as compared with **Lac-2** and **Lac-4**, respectively. The comparison indicates that the parent DAG-lactones probably bind to the enzyme in the sn-2 mode, since its modification caused more severe losses in binding affinity than that of the sn-1 mode. This result also confirmed our previous suggestion that DAG-lactones usually favor the sn-2 binding mode to the crystallographic position of phorbol 13-acetate in the C1b domain.[9]

In view of the importance of the sn-2 (or lactone) ether oxygen and the favorable sn-2 binding mode in DAG-lactones indicated by the above SAR, we examined the possibility that the ether oxygen of the lactone may participate in hydrogen bonding with the enzyme. A molecular modeling study of **Lac-3** indicated that the two oxygens in the lactone may interact with the amide proton of Gly253 in a bifurcated hydrogen bonding mode, the so-called three-centered interaction,[16,17] as shown in Figure 3.[18] Even though the distance between the ring oxygen and the proton was 2.89 Å, which is a little longer than the optimal hydrogen bonding range (1.5–2.5 Å), it would be sufficient for them to participate in the three-centered interaction, once the enzyme conformation is induced.

In summary, we have replaced the sn-1 and sn-2 carbonyl esters of DAG-lactone, identified as a potent lead PK-C ligand, by isosteres including ketone, amide, and alcohol, respectively. None of the compounds surpassed the strong potency of the parent DAG-lactones in terms of their binding affinities to PK-Cα, although the ketone analogue of the sn-1 ester showed comparable activity to the parent DAG-lactone when considering the reduction in lipophilicity. This finding indicated that both esters in DAG-lactones were crucial to bind tightly with the enzyme through favorable hydrogen bonding. The comparative analysis of the sn-1 and sn-2 isosteric

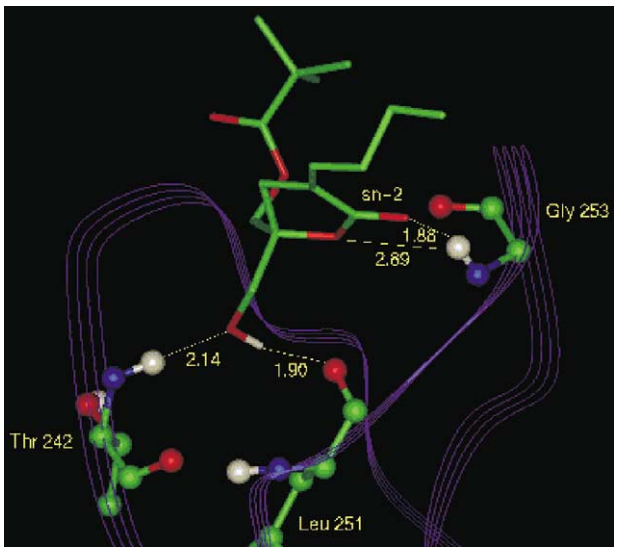


Figure 3. sn-2 binding mode of DAG-lactone (**Lac-3**) with bifurcated hydrogen bonding.

analogues support the conclusion that the parent DAG-lactones bind to the C1-domain of PK-C more favorably in the sn-2 binding mode, as previously speculated.

Experimental

All chemical reagents were commercially available. Melting points were determined on a Melting Point Büchi B-540 apparatus and are uncorrected. Silica gel column chromatography was performed on silica gel 60, 230–400 mesh, Merck. Proton NMR spectra were recorded on a JEOL JNM-LA 300 at 300 MHz. Chemical shifts are reported in ppm units with Me_4Si as a reference standard. Infrared spectra were recorded on a Perkin-Elmer 1710 Series FTIR. Mass spectra were recorded on a VG Trio-2 GC-MS. Elemental analyses were performed with an EA 1110 Automatic Elemental Analyzer, CE Instruments. The syntheses of (5*S*)-5-(hydroxymethyl)-5-[2-(methoxycarbonyl)-(*E*)-ethenyl]-3-{(*E*)-[(*Z*)-9-octadecenyldene]} tetrahydro-2-furanone (**Lac-1**), 5-[(acetyloxy)methyl]-5-hydroxymethyl-3-[(*E*,9*Z*)-9-octadecenylidene]tetrahydro-2-furanone (**Lac-4**) and 5-[(acetyloxy)methyl]-5-hydroxymethyl-3-[(*Z*,9*Z*)-9-octadecenylidene]tetrahydro-2-furanone (**Lac-5**) were described in our previous report.[11,13]

5-Hydroxymethyl-5-pivaloyloxymethyl-3-[(*E*)-tetradecylidene]tetrahydro-2-furanone (Lac-2) and 5-hydroxymethyl-5-pivaloyloxymethyl-3-[(*Z*)-tetradecylidene]tetrahydro-2-furanone (Lac-3). These compounds were prepared by following our procedure described in ref 11.

Lac-2. A colorless oil, ^{1}H NMR ($CDCl_3$) δ 6.77 (m, 1H, >C=CH), 4.29 (d of AB, 1H, J=11.7 Hz, CH_2OCO), 4.14 (d of AB, 1H, J=11.7 Hz, CH_2OCO), 3.68 (ddd of AB, 2H, CH_2OH), 2.83 (m, 1H, H-4a), 2.66 (m, 1H, H-3b), 2.26 (t, 1H, OH), 2.17 (dd of AB, 2H, >C=CH–CH_2), 1.2–1.5 (m, 22H), 1.19 (s, 9H, $C(CH_3)_3$), 0.88 (distorted t, 3H).; IR (neat) 3426 (OH), 1726 (C=O), 1681 cm^{-1}; MS (EI) m/z 424 (M^+).

Lac-2. A colorless oil, ^{1}H NMR ($CDCl_3$) δ 6.25 (m, 1H, >C=CH), 4.28 (d of AB, 1H, J=11.3 Hz, CH_2OCO), 4.13 (d of AB, 1H, J=11.3 Hz, CH_2OCO), 3.66 (ddd of AB, 2H, CH_2OH), 2.90 (m, 1H, H-4a), 2.65–2.85 (m, 3H, H-3b and >C=CH–CH_2), 2.09 (t, 1H, OH), 1.2–1.5 (m, 22H), 1.19 (s, 9H, $C(CH_3)_3$), 0.88 (distorted t, 3H); IR (neat) 3438 (OH), 1737 (C=O), 1681 cm^{-1}; MS (EI) m/z 424 (M^+).

(*S*)-5-(Hydroxymethyl)-5-[2-(methylcarbonyl)-(*E*)-ethenyl]-3-{(*E*)-[(*Z*)-9-octadecenyldene]}tetrahydro-2-furanone (2). A solution of **1** (80 mg, 0.166 mmol) prepared by the method reported in previous literature[12] in CH_2Cl_2 (5 mL) was treated with 1-triphenylphosphoranylidene-2-propanone (106 mg, 0.332 mmol) and stirred for 16 h at room temperature. The mixture was concentrated in vacuo and the residue was purified by flash column chromatography on silica gel with EtOAc/hexanes (1:3) as eluant to give the ketone as an oil (68 mg, 78%). The compound (68 mg, 0.13 mmol) was dissolved in CH_2Cl_2 (4 mL) and cooled to −78 °C. The solution was treated with boron trichloride (1.0 M in CH_2Cl_2, 0.65 mL, 0.65 mmol) slowly and stirred for 90 min at −78 °C. The reaction mixture was quenched with saturated $NaHCO_3$ solution and immediately partitioned between ether and pH 7 buffer solution. The organic layer was washed with H_2O, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel with EtOAc/hexanes (2:3 to 1:1) as eluant to give **2** as an oil (44 mg, 77%): $[\alpha]_D$ +17.5 (*c* 0.32, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 6.76 (m, 1H, >C=CH), 6.73 (d, 1H, J=15.9 Hz, CH=CHCOMe), 6.39 (d, 1H, J=15.9 Hz, CH=CHCOMe), 5.33 (m, 2H, CH_2CH=$CHCH_2$), 3.77 (d of AB, 1H, J=12.1 Hz, CH_2OH), 3.64 (d of AB, 1H, J=12.1 Hz, CH_2OH), 3.05 (m, 1H, H-4a), 2.68 (m, 1H, H-4b), 2.26 (s, 3H, $COCH_3$), 2.14 (m, 2H, >C=CH-CH_2), 1.9–2.05 (m, 4H, CH_2CH=$CHCH_2$), 1.1–1.6 (m, 22H), 0.86 (distorted t, 3H); ^{13}C NMR ($CDCl_3$) δ 197.18, 169.66, 143.04, 142.87, 130.04, 129.65, 129.51, 124.93, 83.76, 66.81, 38.53, 38.45, 32.35, 31.87, 30.34, 29.73, 29.68, 29.49, 29.29, 29.17, 29.12, 29.00, 28.65, 28.00, 27.20, 27.12, 26.49, 26.42, 22.66, 14.09; IR (neat) 3383 (OH), 1731 and 1681 (C=O), 1673 (C=C) cm^{-1}. Anal. calcd for $C_{27}H_{44}O_4$: C, 74.95; H, 10.25. Found: C, 73.86; H, 10.29.

(*S*)-5-(Hydroxymethyl)-5-[(acetylamino)methyl]-3-{(*E*)-[(*Z*)-9-octadecenyldene]}tetrahydro-2-furanone (3). A cooled solution of **1** (145 mg, 0.3 mmol) in MeOH (6 mL) in an ice-bath was treated portionwise with sodium borohydride (46 mg, 1.2 mmol) and stirred for 1 h at 0 °C. The reaction mixture was quenched with excess acetone and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel with EtOAc/hexanes (1:2) as eluant to give the alcohol (125 mg, 86%). A solution of the above alcohol (96 mg, 0.2 mmol) in CH_2Cl_2 (5 mL) was treated with triethylamine (0.084 mL, 0.6 mmol) and methanesulfonyl

chloride (0.03 mL, 0.4 mmol) and stirred for 2 h. The mixture was concentrated in vacuo and the residue was purified by flash column chromatography on silica gel with EtOAc/hexanes (1:2) as eluant to give the mesylate. The mesylate was dissolved in DMF (3 mL) and treated with sodium azide (65 mg, 1 mmol). After being stirred for 16 h at 100 °C, the mixture was diluted with H_2O and extracted with EtOAc several times. The combined organic layers were washed with H_2O and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel with EtOAc/hexanes (1:2) as eluant to give the azide (86 mg, 84%).

^{1}H NMR ($CDCl_3$) δ 6.75 (m, 1H, >C=CH), 5.32 (m, 2H, $CH_2\underline{CH}$=$\underline{CH}CH_2$), 4.55 (s, 2H, $PhCH_2O$), 3.60 (q of AB, 2H, $\underline{CH_2}N_3$), 3.55 (s, 2H, $BnOCH_2$), 3.05 (m, 1H, H-4a), 2.65 (m, 1H, H-4b), 2.15 (m, 2H, >C=CH-$\underline{CH_2}$), 1.9–2.05 (m, 4H, $\underline{CH_2}CH$=$CHC\underline{H_2}$), 1.1–1.6 (m, 22H), 0.86 (distorted t, 3H).

A mixture of the azide (82 mg, 0.16 mmol), acetic anhydride (0.06 mL, 0.64 mmol) and Lindler's catalyst (16 mg) in EtOAc (5 mL) was hydrogenated under a balloon of hydrogen for 2 h, filtered and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel with EtOAc/hexanes (2:1) as eluant to give the acetamide (66 mg, 78%) as an oil, which was debenzylated by following the debenzylation method described for the synthesis of **2** and purified by flash column chromatography on silica gel with EtOAc/hexanes (5:1) to give **3** as an oil in 92% yield: $[\alpha]_D = +12.5$ (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 6.77 (m, 1H, >C=CH), 6.17 (bt, 1H, NH), 5.33 (m, 2H, $CH_2\underline{CH}$=$\underline{CH}CH_2$), 3.99 (d of AB, 1H, J=8.1 Hz, $NHC\underline{H_2}$), 3.93 (d of AB, 1H, J=8.1 Hz, $NHCH_2$), 3.57 (d of AB, 1H, J=12.0 Hz, CH_2OH), 3.29 (d of AB, 1H, J=12.0 Hz, CH_2OH), 2.9–3.1 (m, 1H, H-4a and OH), 2.48 (m, 1H, H-4b), 2.05 (s, 3H, $NHCOCH_3$), 1.9–2.2 (m, 6H, >C=CH–$\underline{CH_2}$ and $\underline{CH_2}CH$=$CHC\underline{H_2}$), 1.1–1.6 (m, 22H), 0.86 (distorted t, 3H); ^{13}C NMR ($CDCl_3$) δ 172.43, 170.00, 143.23, 130.03, 129.66, 125.29, 84.12, 63.38, 44.26, 34.38, 31.87, 31.80, 31.68, 30.30, 29.73, 29.67, 29.49, 29.29, 29.17, 29.12, 29.00, 28.85, 28.05, 27.20, 27.13, 26.69, 26.63, 22.89, 22.65, 14.09; IR (neat) 3384 (OH), 1730 and 1680 (C=O), 1665 (C=C) cm^{-1}. Anal. calcd for $C_{26}H_{45}O_4N$: C, 71.68; H, 10.41; N, 3.22. Found: C, 71.94; H, 10.44; N. 3.20.

Diethyl 3-cyclopentene-1,1-dicarboxylate (4). To a sodium ethoxide solution prepared from sodium (2.3 g, 100 mmol) and ethanol (40 mL) was added diethylmalonate (6 g, 37 mmol) followed by 1,4-dichloro-2-butene (3.94 mL, 37 mmol). After being refluxed for 16 h, the reaction mixture was cooled and concentrated in vacuo. The residue was extracted with ether several times. The combined organic layers were washed with H_2O and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel with EtOAc/hexanes (1:4) as eluant to give **4** as an oil (5.8 g, 75%); ^{1}H NMR ($CDCl_3$) δ 5.60 (s, 2H, CH=CH), 4.20 (q, 4H, 2×$CO_2C\underline{H_2}CH_3$), 3.01 (s, 4H, 2×CH_2), 1.25 (m, 6H, 2×$CO_2CH_2C\underline{H_3}$).

8,8-dimethyl-7,9-dioxaspiro[4.5]dec-2-ene (5). A cooled solution of lithium aluminum hydride (2.07 g, 54.8 mmol) at 0 °C in diethyl ether (50 mL) was dropwise treated with a solution of **4** (2.9 g, 13.7 mmol) in diethyl ether (20 mL). After being stirred for 2 h at room temperature, the reaction mixture was cooled in an ice-bath and quenched with H_2O (2.07 mL), 15% NaOH (2.07 mL) and H_2O (6.21 mL), successively. The suspension was stirred for 1 h, filtrated with ether and the filtrate was concentrated in vacuo. The residue was purified by flash column chromatography on silica gel with EtOAc/hexanes (1:1) as eluant to give the diol as a solid (1.4 g, 84%); ^{1}H NMR ($CDCl_3$) δ 5.56 (s, 2H, CH=CH), 3.81 (bs, 2H, 2×OH), 3.58 (s, 4H, 2×CH_2OH), 2.12 (s, 4H, 2×CH_2).

The diol (1.474 g, 11.5 mmol) was dissolved in cooled DMF (5 mL) at 0 °C and treated with 2-methoxypropene (2.2 mL, 23 mmol) and a catalytic amount of *p*-TsOH. After being stirred for 4 h at room temperature, the reaction mixture was treated with solid $NaHCO_3$ and extracted with EtOAc several times. The combined organic layers were washed with H_2O and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel with EtOAc/hexanes (1:4) as eluant to give **5** as an oil (1.88 g, 98%); ^{1}H NMR ($CDCl_3$) δ 5.58 (s, 2H, CH=CH), 3.63 (s, 4H, 2×OCH_2), 2.23 (s, 4H, 2×CH_2), 1.40 (s, 6H, 2×CH_3).

3-[(4-Methoxybenzyl)oxy]-8,8-dimethyl-7,9-dioxaspiro[4.5]-decan-2-ol (6). A solution of **5** (1.89 g, 10 mmol) in acetone (20 mL) was treated with 4-methylmorpholine N-oxide (2.34 g, 20 mmol) and a catalytic amount of osmium tetroxide and stirred for 16 h at room temperature. The reaction mixture was quenched by aqueous $Na_2S_2O_3$ solution and extracted with EtOAc several times. The combined organic layers were washed with H_2O and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel with EtOAc/hexanes (1:1) as eluant to give the corresponding diol as a solid (2.87 g, 71%); ^{1}H NMR ($CDCl_3$) δ 4.07 (m, 2H, 2×$C\underline{H}OH$), 3.70 (s, 2H, OCH_2), 3.52 (s, 2H, OCH_2), 3.09 (bs, 2H, 2×OH), 1.78 (dd, 2H, J=6.1, 14 Hz, >$C\underline{H_2}CH$), 1.66 (dd, 2H, J=5.1, 14 Hz, >$C\underline{H_2}CH$), 1.40 (s, 6H, 2×CH_3).

A solution of the above diol (1.21 g, 6.0 mmol) in THF (4 mL) was treated with NaH (0.24 g, 6.0 mmol) and stirred for 30 min at room temperature. The mixture was treated with *p*-methoxybenzyl chloride (0.8 mL, 6.0 mmol) followed by tetrabutylammonium iodide (1.1 g, 3.0 mmol) and stirred for 20 h at room temperature. The reaction mixture was concentrated in vacuo and diluted with CH_2Cl_2. The organic layers were washed with H_2O and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel with EtOAc/hexanes (1:1) as eluant to give **6** as an oil (1.01 g, 52%); ^{1}H NMR ($CDCl_3$) δ 7.27 (d, J=8.4 Hz, 2H, Ar), 6.90 (d, J=8.4 Hz, 2H, Ar), 4.57 (d, J=11.3 Hz, 1H, OCH_2Ar), 4.45 (d, J=11.3 Hz, 1H, OCH_2Ar), 4.12 (m, 1H,

CHOPMB), 3.8–3.9 (m, 4H, CHOH and OCH_3), 3.74 (dd of AB, 2H, CH_2O), 3.54 (dd of AB, 2H, CH_2O), 2.44 (d, 1H, OH), 1.90 (ddd of AB, 2H, $CH_2CHOPMB$), 1.63 (ddd of AB, 2H, CH_2CHOH), 1.43 (s, 6H, $2\times CH_3$).

3-[(4-Methoxybenzyl)oxy]-8,8-dimethyl-7,9-dioxaspiro[4.5]decan-2-one (7). A suspension of **6** (0.818 g, 2.54 mmol) and 4 Å molecular sieve (1.095 g) in CH_2Cl_2 (8 mL) was treated with pyridinium chlorochromate (1.095 g, 5.08 mmol) and stirred for 12 h at room temperature. The mixture was quenched with ether and Celite and stirred for 30 min. It was filtrated through a silica gel pad with an aid of ether and the filtrate was concentrated in vacuo. The residue was purified by flash column chromatography on silica gel with EtOAc/hexanes (1:2) as eluant to give **7** as a solid (0.789 g, 97%); ^{1}H NMR ($CDCl_3$) δ 7.27 (d, $J=8.4$ Hz, 2H, Ar), 6.87 (d, $J=8.4$ Hz, 2H, Ar), 4.74 (d, $J=11.5$ Hz, 1H, OCH_2Ar), 4.56 (d, $J=11.5$ Hz, 1H, OCH_2Ar), 3.86 (t, 1H, $J=7.8$ Hz, CHOPMB), 3.80 (s, 3H, OCH_3), 3.72 (dd of AB, 2H, CH_2O), 3.57 (dd of AB, 2H, CH_2O), 2.35 (s, 2H, CH_2CO), 2.18 (dd of AB, 1H, $CH_2CHOPMB$), 1.68 (dd of AB, 1H, $CH_2CHOPMB$), 1.44 (s, 3H, CH_3), 1.40 (s, 3H, CH_3).

3-[(E or Z)-Tetradecylidene]-8,8-dimethyl-7,9-dioxaspiro[4.5]-decan-2-one (8, 9). A mixture of **7** (1.24 g, 3.88 mmol) and tetradecyl triphenylphosphonium bromide (4.18 g, 7.76 mmol) was dried overnight under vacuum and dissolved in benzene (30 mL). The suspension was refluxed and treated with potassium *tert*-butoxide in THF until starting material was consumed as determined by TLC. The mixture was cooled and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel with EtOAc/hexanes (1:10) as eluant to give the corresponding alkene as an oil (1.39 g, 72%). A solution of the above alkene (1.188 g, 2.37 mmol) in CH_2Cl_2-H_2O (19 mL, 18:1) was treated with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (0.808 g, 3.56 mmol) and stirred for 2 h at room temperature. The reaction mixture was quenched by aqueous $Na_2S_2O_3$ and extracted with CH_2Cl_2 several times. The combined organic layers were washed with H_2O and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel with EtOAc/hexanes (1:10) as eluant to give the corresponding alcohol as an oil (0.603 g, 67%). A solution of the above alcohol (0.393 g, 1.033 mmol) in CH_2Cl_2 (15 mL) was treated with 4 Å molecular sieve (0.668 g) and pyridinium chlorochromate (0.668 g, 3.1 mmol) and stirred for 6 h at room temperature. The mixture was quenched with ether and Celite, stirred for an addition 30 min, filtered through a short pad of silica gel and the filtrate was concentrated in vacuo. The residue was purified by flash column chromatography on silica gel with EtOAc/hexanes (1:10) as eluant to give **8** (*E*-isomer, 0.278 g, 27%) and **9** (*Z*-isomer, 0.455 g, 44%) as an oil, respectively.

8. *E*-Isomer, ^{1}H NMR($CDCl_3$) δ 6.63 (t, 1H, $J=7.5$ Hz, $>C=CH$), 3.66 (dd of AB, 4H, $2\times OCH_2$), 2.61 (s, 2H, H-1), 2.29 (s, 2H, H-4), 2.16 (dd of AB, 2H, $>C=CH\text{-}CH_2$), 1.47 (s, 3H, CH_3), 1.45 (s, 3H, CH_3), 1.25–1.3 (m, 22H), 0.90 (distorted t, 3H).

9. *Z*-Isomer, ^{1}H NMR($CDCl_3$) δ 6.03 (t, 1H, $J=7.3$ Hz, $>C=CH$), 3.68 (ddd of AB, 4H, $2\times OCH_2$), 2.6–2.7 (m, 4H, H-1 and $>C=CH–CH_2$), 2.27 (s, 2H, H-4), 1.47 (s, 3H, CH_3), 1.45 (s, 3H, CH_3), 1.25–1.3 (m, 22H), 0.90 (distorted t, 3H).

4-Hydroxymethyl-4-pivaloyloxymethyl-2-[(*E*)-tetradecylidene]cyclopentanone (10) and 4-hydroxymethyl-4-pivaloyloxymethyl-2-[(*Z*)-tetradecylidene]cyclopentanone (11). A solution of 8 (188 mg, 0.5 mmol) and *p*-TsOH (10 mg, 0.05 mmol) in MeOH (4 mL) was stirred for 2 h at room temperature. The mixture was neutralized with solid $NaHCO_3$, filtered and the filtrate was concentrated in vacuo. The residue was purified by flash column chromatography on silica gel with EtOAc/hexanes (3:1) as eluant to give the diol as an oil (122 mg, 70%). The diol (122 mg, 0.36 mmol) was dissolved in CH_2Cl_2 (6 mL), treated with pyridine (0.06 mL, 0.72 mmol) and stirred for 2 h at room temperature. The mixture was treated with pivaloyl chloride (0.44 mL, 0.36 mmol), stirred for 2 h at room temperature and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel with EtOAc/hexanes (1:4) as eluant to give 10 as an oil (114 mg, 75%).

10. E-Isomer; ^{1}H NMR($CDCl_3$) δ 6.63 (m, 1H, $>C=CH$), 4.18 (d of AB, 1H, $J=11.5$ Hz, CH_2OCO), 4.02 (d of AB, 1H, $J=11.5$ Hz, CH_2OCO), 3.44 (s, 2H, CH_2OH), 2.58 (m, 1H, H-3a), 2.44 (m, 1H, H-3b), 2.32 (s, 2H, H-5), 2.13 (dd of AB, 2H, $>C=CH–CH_2$), 1.25–1.5 (m, 22H), 1.21 (s, 9H, $C(CH_3)_3$), 0.88 (distorted t, 3H); IR (neat) 3443 (OH), 1731, 1715 (C=O) cm^{-1}; MS (EI) m/z 423 (M^+). Anal. calcd for $C_{26}H_{46}O_4$: C, 73.89; H, 10.97; Found: C, 74.02; H, 10.99.

11. Z-Isomer, prepared from **9** by following the procedure described for the synthesis of **10** in 53% yield; ^{1}H NMR($CDCl_3$) δ 6.60 (m, 1H, $>C=CH$), 4.21 (d of AB, 1H, $J=11.2$ Hz, CH_2OCO), 4.00 (d of AB, 1H, $J=11.2$ Hz, CH_2OCO), 3.41 (dd of AB, 2H, CH_2OH), 2.63 (t, 2H, H-5), 2.4–2.55 (m, 4H, H-3 and $>C=CH–CH_2$), 1.25–1.6 (m, 22H), 1.21 (s, 9H, $C(CH_3)_3$), 0.88 (distorted t, 3H); IR (neat) 3443 (OH), 1730, 1715 (C=O) cm^{-1}; MS (EI) m/z 423 (M^+). Anal. calcd for $C_{26}H_{46}O_4$: C, 73.89; H, 10.97; Found: C, 74.04; H, 11.00.

(*rel*-1*R*,4*S*)-4-Hydroxymethyl-4-pivaloyloxymethyl-2-[(*E*)-tetradecylidene]cyclopentanol (12) and (*rel*-1*S*,4*R*)-4-hydroxymethyl-4-pivaloyloxymethyl-2-[(*Z*)-tetradecylidene]cyclopentanol (13). A cooled solution of **10** (105 mg, 0.24 mmol) in CH_2Cl_2–MeOH (8 mL, 2:1) at 0 °C was treated with cerium(III) chloride heptahydrate (96 mg, 0.264 mmol) followed by sodium borohydride (10 mg, 0.264 mmol) and stirred for 30 min at 0 °C. The reaction mixture was quenched by 1 N HCl and extracted with CH_2Cl_2 several times. The combined

organic layers were washed with H_2O and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel with EtOAc/hexanes (1:2) as eluant to give **12** (36 mg, 35%) and **13** (30 mg, 30%) as white solids, respectively.

12. Mp 40 °C; ^{1}H NMR ($CDCl_3$) δ 5.55 (m, 1H, >C=CH), 4.51 (m, 1H, CHOH), 4.20 (dd of AB, 2H, J = 9.7 Hz, CH_2OCO), 3.29 (d, 2H, J = 5.6 Hz, CH_2OH), 2.54 (t, 1H, OH), 2.24 (dd of AB, 2H, H-3), 1.99 (dd of AB, 2H, >C=CH–CH_2), 1.90 (dd of AB, 2H, J = 6.8, 13.9 Hz, H-5), 1.25–1.6 (m, 22H), 1.21 (s, 9H, $C(CH_3)_3$), 0.88 (distorted t, 3H); IR (KBr) 3440, 1730, 1711 cm^{-1}; MS (EI) m/z 424 (M^+). Anal. calcd for $C_{26}H_{48}O_4$: C, 73.54; H, 11.39; Found: C, 73.79; H, 11.41.

13. Mp 40 °C; ^{1}H NMR ($CDCl_3$) δ 5.56 (m, 1H, >C=CH), 4.49 (m, 1H, CHOH), 3.96 (dd of AB, 2H, J = 11.2 Hz, CH_2OCO), 3.54 (dd of AB, 2H, J = 11 Hz, CH_2OH), 2.44 (d of AB, 1H, H-3a), 2.14 (d of AB, 1H, H-3b), 1.9–2.0 (m, 4H, >C=CH–CH_2 and H-5), 1.25–1.65 (m, 22H), 1.21 (s, 9H, $C(CH_3)_3$), 0.88 (distorted t, 3H); IR (KBr) 3439, 1731, 1710 cm^{-1}; MS (EI) m/z 424 (M^+). Anal. calcd for $C_{26}H_{48}O_4$: C, 73.54; H, 11.39; Found: C, 73.82 ; H, 11.43.

***tert*-Butyl *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]-carbamate (15).** A solution of trishydroxymethylaminomethane (**14**) (6.0 g, 50 mmol) in a mixture of triethylamine and methanol (1:9, 150 mL) was treated with di-*tert*-butyl dicarbonate (13.1 g, 60 mmol) and stirred at room temperature for 3 h. The reaction mixture was concentrated in vacuo, diluted with H_2O and extracted with EtOAc three times. The combined organic layer was washed with H_2O and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel with CH_2Cl_2/MeOH (9:1) as eluant to give **15** as a solid (8.06 g, 72%): mp 147 °C; ^{1}H NMR ($CDCl_3$) δ 5.50 (bs, 1H, NH), 3.68 (m, 6H, 3×CH_2), 3.33 (bs, 3H, 3×OH), 1.45 (s, 9H, $C(CH_3)_3$).

***tert*-Butyl *N*-[5-(hydroxymethyl)-2,2-dimethyl-1,3-dioxan-5-yl]carbamate (16).** A solution of **15** (8.06 g, 36 mmol) in acetone (150 mL) was treated with a catalytic amount of *p*-toluene sulfonate and stirred for 18 h at room temperature. The reaction mixture was alkalinized with solid $NaHCO_3$, filtered and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel with EtOAc/hexanes (1:1) as eluant to give **16** as a solid (7.18 g, 76%): mp = 99 °C; ^{1}H NMR ($CDCl_3$) δ 5.34 (s, 1H, NH), 3.85 (d, 2H, J = 11.5 Hz, CH_2O), 3.80 (d, 2H, J = 11.5 Hz, CH_2O), 3.72 (s, 2H, CH_2OH), 1.41–1.50 (m, 15H, $C(CH_3)_3$ and $C(CH_3)_2$); ^{13}C NMR ($CDCl_3$) δ 156.43, 98.75, 80.45, 64.71, 64.38, 53.32, 28.33, 26.81, 20.31; IR (KBr) 3421 (OH), 1715 (C=O) cm^{-1}.

***tert*-Butyl *N*-(5-formyl-2,2-dimethyl-1,3-dioxan-5-yl)carbamate (17).** A solution of **16** (6.3 g, 24 mmol) in CH_2Cl_2 (100 mL) was treated with 4 Å molecular sieve (12.6 g) and stirred for 30 min at room temperature. To the mixture was added pyridinium chloroformate (10.4 g, 48 mmol) portionwise. After stirring for 12 h at room temperature, the reaction mixture was treated with Celite and ether, stirred for 30 min and filtered through a short pad of silica gel. The filtrate was concentrated in vacuo and the residue was purified by flash column chromatography on silica gel with EtOAc/hexanes (1:4) as eluant to give **17** as a solid (4.90 g, 78%): mp 62 °C; ^{1}H NMR ($CDCl_3$) δ 9.63 (s, 1H, CHO), 5.57 (bs, 1H, NH), 4.07 (d, 2H, J = 11.7 Hz, CH_2O), 3.95 (d, 2H, J = 11.7 Hz, CH_2O), 1.46–1.52 (m, 15H, $C(CH_3)_3$ and $C(CH_3)_2$); ^{13}C NMR ($CDCl_3$) δ 199.25, 155.40, 98.67, 80.82, 62.55, 59.77, 28.15, 27.11, 19.58.

Methyl 3-{5-[(*tert*-butoxycarbonyl)amino]-2,2-dimethyl-1,3-dioxan-5-yl}propanoate (18). A solution of **17** (4.45 g, 17 mmol) and methyl (triphenylphosphoranylidene)acetate (8.53 g, 25.5 mmol) in CH_2Cl_2 (150 mL) was stirred for 20 h at room temperature. The mixture was concentrated in vacuo and the residue was purified by flash column chromatography on silica gel with EtOAc/hexanes (1:4) as eluant to give the α,β-unsaturated methyl ester as a white solid (4.27 g, 80%): mp 85 °C; ^{1}H NMR ($CDCl_3$) δ 6.93 (d, 1H, J = 16.1 Hz, CH=$CHCO_2CH_3$), 5.98 (d, J = 16.1 Hz, 1H, CH=$CHCO_2CH_3$), 5.20 (bs, 1H, NH), 3.88 (t, 4H, J = 14.2 Hz, 2×CH_2O), 3.74 (s, 3H, CO_2CH_3), 1.46–1.52 (m, 15H, $C(CH_3)_3$ and $C(CH_3)_2$).

The above compound (3.96 g, 12.6 mmol) was dissolved in EtOAc (40 mL), treated with 10% Pd/C (0.4 g) and hydrogenated under a balloon of hydrogen for 5 h. The reaction mixture was filtered through Celite and the filtrate was concentrated in vacuo. The residue was purified by flash column chromatography on silica gel with EtOAc/hexanes (1:3) as eluant to give **18** as a white solid (3.6 g, 90%); mp 65 °C; ^{1}H NMR ($CDCl_3$) δ 4.95 (bs, 1H, NH), 3.78 (d, J = 14.4 Hz, 2H, CH_2O), 3.57 (d, J = 14.4 Hz, 2H, CH_2O), 3.56 (s, 3H, CO_2CH_3), 2.23 (t, J = 7.6 Hz, 2H, $CH_2CO_2CH_3$), 1.93 (t, J = 8.3 Hz, 2H, $CH_2CH_2CO_2CH_3$), 1.34–1.40 (m, 15H, $C(CH_3)_3$ and $C(CH_3)_2$); ^{13}C NMR ($CDCl_3$) δ 173.45, 154.56, 98.03, 78.94, 65.50, 51.28, 50.81, 28.02, 27.42, 26.47, 26.21, 20.21; IR (KBr) 1735 and 1715 (C=O) cm^{-1}.

8,8-Dimethyl-7,9-dioxa-1-azaspiro[4.5]decan-2-one (19). A solution of **18** (3.0 g, 9.45 mmol) in methanol (100 mL) was treated with 28% sodium methoxide (1.62 mL, 28.35 mmol) and refluxed for 12 h. After cooling, the reaction mixture was concentrated in vacuo. The residue was dissolved in ethyl acetate, filtrated with Celite and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel with EtOAc/MeOH (9:1) as eluant to give **19** as a white solid (1.34 g, 77%); mp 174 °C; ^{1}H NMR ($CDCl_3$) δ 6.41 (bs, 1H, NH), 3.84 (d, 2H J = 11.5 Hz, CH_2O), 3.63 (d, 2H, J = 11.5 Hz, CH_2O), 2.41 (t, 2H, J = 7.8 Hz, CH_2CONH), 1.77 (t, 2H, J = 8.8 Hz, CH_2CH_2CONH), 1.46 (s, 3H, CH_3), 1.42 (s, 3H, CH_3); IR (KBr) 3187 (NH), 1715, 1650 (C=O) cm^{-1}.

***tert*-Butyl 8,8-dimethyl 2-oxo-7,9-dioxa-1-azaspiro[4.5] decane-1-carboxylate (20).** A solution of **19** (0.648 g, 3.5 mmol), di-*tert*-butyl dicarbonate (2.3 g, 10.5 mmol), triethylamine (2.5 mL, 17.5 mmol) and 4-dimethyl aminopyridine (0.085 g, 0.7 mmol) in THF (10 mL) was refluxed for 24 h. The reaction mixture was cooled and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel with EtOAc/hexanes (1:2) as eluant to give **20** as a white solid (0.572 g, 57%): mp 106 °C; ^{1}H NMR ($CDCl_3$) δ 4.62 (d, 2H, J = 11 Hz, OCH_2), 3.46 (d, 2H, J = 11 Hz, OCH_2), 2.48 (t, 2H, J = 8.3 Hz, CH_2CONH), 2.28 (t, 2H, J = 8 Hz, CH_2CH_2CONH), 1.55 (m, 12H, $C(CH_3)_3$ and $(CH_3)_2C$), 1.41 (s, 3H, $(CH_3)_2C$); ^{13}C NMR ($CDCl_3$) δ 174.43, 150.27, 98.60, 83.80, 64.28, 60.41, 29.68, 28.16, 28.05, 27.68, 19.42; IR (KBr) 1754 and 1709 (C=O) cm^{-1}.

***tert*-Butyl-3-[(*E*,9*Z*)-9-octadecenylidene]-8,8-dimethyl-2-oxo-7,9-dioxa-1-azaspiro[4.5]decane-1-carboxylate (21) and *tert*-butyl-3-[(*Z*,9*Z*)-9-octadecenylidene]-8,8-dimethyl-2-oxo-7,9-dioxa-1-azaspiro[4.5]decane-1-carboxylate (22).** A solution of **20** (0.574 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 stirring for 30 min, a solution of oleyl aldehyde (0.7 g, 2.6 mmol) in THF (2 mL) was added and stirring was continued for 4 h at −78 °C. The reaction mixture was quenched with a solution of ammonium chloride and diluted with ether. The organic layer was washed with H_2O, dried over $MgSO_4$, 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 lactam (0.785 g, 71%) as an oil. The compound was dissolved in CH_2Cl_2 (8 mL), cooled to 0 °C, and treated with triethylamine (0.82 mL, 5.68 mmol) and methanesulfonyl chloride (0.22 mL, 2.84 mmol). After stirring for 3 h at room temperature, the reaction mixture was cooled to 0 °C and 1,8-diazabicyclo[5,4,0]undec-7-ene (0.637 mL, 4.26 mmol) was added. After refluxing for 12 h, the reaction mixture was concentrated in vacuo. The residue was diluted with ether, washed with 1 N HCl and H_2O, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel with EtOAc/hexanes (1:10) as eluant to give **21** (0.425 g, 56%) and **22** (0.228 g, 30%), respectively, as oils.

21. E-Isomer, ^{1}H NMR ($CDCl_3$) δ 6.74 (m, 1H, >C=CH), 5.35 (m, 2H, CH=CH), 4.74 (d, 2H, J = 11 Hz, OCH_2C), 3.37 (d, 2H, J = 11 Hz, OCH_2C), 2.91 (bs, 2H, H-4), 2.18 (m, 2H, >C=CHC$\underline{H}_2$), 2.0 (m, 4H, C$\underline{H}_2$CH=CHC$\underline{H}_2$), 1.58 (s, 3H, $C(CH_3)_2$), 1.56 (s, 9H, $NCO_2C(CH_3)_3$), 1.44 (s, 3H, $C(CH_3)_2$), 1.25–1.5 (m, 22H), 0.88 (distorted t, 3H); ^{13}C NMR ($CDCl_3$) δ 167.37, 151.07, 140.13, 130.01, 129.77, 128.34, 98.46, 83.65, 64.91, 57.59, 33.53, 31.92, 29.78, 29.74, 29.53, 29.48, 29.32, 29.20, 28.61, 28.33, 28.12, 27.24, 27.19, 22.69, 19.16, 14.12; IR (neat) 1746 and 1714 (C=O), 1457 cm^{-1}.

22. Z-Isomer, ^{1}H NMR ($CDCl_3$) δ 6.11 (m, 1H, >C=CH), 5.35 (m, 2H, CH=CH), 4.67 (d, 2H, J = 11.2 Hz, OCH_2C), 3.40 (d, 2H, J = 11.2 Hz, OCH_2C), 2.91 (m, 2H, H-4), 2.72 (m, 2H, >C=CHC$\underline{H}_2$), 2.0 (m, 4H, C$\underline{H}_2$CH=CHC$\underline{H}_2$), 1.56 (bs, 12H, $NCO_2C(CH_3)_3$ and $C(CH_3)_2$), 1.41 (s, 3H, $C(CH_3)_2$), 1.25–1.5 (m, 22H), 0.88 (distorted t, 3H).

^{13}C NMR ($CDCl_3$) δ 166.96, 151.05, 144.55, 129.92, 129.81, 126.24, 98.44, 83.56, 64.56, 57.45, 37.08, 31.89, 29.75, 29.51, 29.38, 29.30, 29.22, 28.49, 28.14, 27.65, 27.19, 22.67, 19.23, 14.10; IR (neat) 1739 and 1714 (C=O), 1456 cm^{-1}.

5,5-Bis(hydroxymethyl)-3-[(*E*,9*Z*)-9-octadecenylidene]-2-pyrrolidinone (23). A solution of **21** (0.107 g, 0.2 mmol) in THF (2 mL) and H_2O (0.5 mL) was treated with trifluoroacetic acid (0.25 mL) at 0 °C, and stirred for 12 h at room temperature. The reaction mixture was cooled and alkalinized with solid $NaHCO_3$. The mixture was filtrated and the filtrate was concentrated in vacuo. The residue was purified by flash column chromatography on silica gel with EtOAc as eluant to give **23** (0.057 g, 72%) as a white solid: mp 37 °C; ^{1}H NMR (acetone-d_6) δ 7.48 (bs, 1H, NH), 6.32 (m, 1H, >C=CH), 5.34 (m, 2H, CH=CH), 3.60 (d, 2H, J = 11 Hz, CH_2OH), 3.53 (d, 2H, J = 11 Hz, CH_2OH), 2.53 (m, 2H, H-4), 1.98–2.15 (m, 6H, >C=CHC$\underline{H}_2$ and C$\underline{H}_2$CH=CHC$\underline{H}_2$), 1.25–1.5 (m, 22H), 0.87 (distorted t, 3H).

5,5-Bis(hydroxymethyl)-3-[(Z,9Z)-9-octadecenylidene]-dihydro-1H-pyrrol-2-one (24). This compound was prepared from **22** by following the above procedure in 79% yield as a white solid: mp 39 °C; ^{1}H NMR (acetone-d_6) δ 6.70 (bs, 1H, NH), 5.77 (m, 1H, >C=CH), 5.30 (m, 2H, CH=CH), 3.48 (m, 4H, CH_2OH), 2.53 (m, 2H, H-4), 1.98–2.15 (m, 6H, >C=CHC$\underline{H}_2$ and C$\underline{H}_2$CH=CHC$\underline{H}_2$), 1.25–1.5 (m, 22H), 0.87 (distorted t, 3H).

{2-(Hydroxymethyl)-4-[(*E*,9*Z*)-9-octadecenylidene]-5-oxo-2-pyrrolidinyl}methyl acetate (25). A solution of **23** (0.126 g, 0.32 mmol) and pyridine (0.05 mL, 0.64 mmol) in CH_2Cl_2 (6 mL) was stirred for 2 h at room temperature, cooled to 0 °C, and treated with acetyl chloride (0.023 mL, 0.32 mmol). After stirring for 16 h at room temperature, the mixture was concentrated in vacuo and the residue was purified by flash column chromatography on silica gel with EtOAc/MeOH (9:1) as eluant to give **25** (0.072 g, 52%) as an oil: ^{1}H NMR ($CDCl_3$) δ 6.53 (m, 1H, >C=CH), 6.07 (bs, 1H, NH), 5.35 (m, 2H, CH=CH), 4.22 (d, 1H, J = 11.2 Hz, $AcOCH_2$), 4.07 (d, 1H, J = 11.2 Hz, $AcOCH_2$), 3.56 (d, 1H, J = 11 Hz, CH_2OH), 3.48 (d, 1H, J = 11 Hz, CH_2OH), 2.65 (m, 2H, >C=CHC$\underline{H}_2$), 2.51 (m, 2H, H-4), 1.9–2.05 (m, 4H, C$\underline{H}_2$CH=CHC$\underline{H}_2$), 1.2–1.4 (m, 22H), 0.83 (distorted t, 3H); ^{13}C NMR ($CDCl_3$) δ 171.27, 170.62, 135.98, 130.03, 129.75, 125.03, 66.28, 65.58, 59.52, 32.21, 30.45, 29.77, 29.75, 29.53, 29.46, 29.34, 29.21, 27.24, 27.19, 23.43, 22.69, 20.75, 14.12.; IR (neat) 3438 (OH), 1745 and 1666 (C=O), 1456 cm^{-1}; MS (EI) m/z 435 (M^+). Anal. calcd for $C_{26}H_{45}NO_4$: C, 71.68; H, 10.41; N, 3.22. Found: C, 72.12; H, 10.45; N, 3.19.

{5-(Hydroxymethyl)-3-[(*Z*,9*Z*)-9-octadecenylidene]-2-oxotetrahydro-3H-pyrrol-5yl}methyl acetate (26). This compound was prepared from **24** by following the above

procedure in 50% yield as an oil; ^{1}H NMR ($CDCl_3$) δ 6.49 (bs, 1H, NH), 5.93 (m, 1H, >C=CH), 5.34 (m, 2H, CH=CH), 4.19 (d, 1H, J = 11.2 Hz, $AcOCH_2$), 4.05 (d, 1H, J = 11.2 Hz, $AcOCH_2$), 3.56 (d, 1H, J = 11.5 Hz, CH_2OH), 3.47 (d, 1H, J = 11.5 Hz, CH_2OH), 2.70 (m, 2H, >C=CHC$\underline{H}_2$), 2.58 (m, 2H, H-4), 2.10 (s, 3H, CH_3CO_2), 1.9–2.1 (m, 4H, C$\underline{H}_2$CH=CHC$\underline{H}_2$), 1.2–1.5 (m, 22H), 0.88 (distorted t, 3H); ^{13}C NMR ($CDCl_3$) δ 171.30, 171.01, 140.39, 129.93, 129.86, 125.03, 66.11, 65.42, 59.13, 34.02, 32.21, 31.91, 29.77, 29.70, 29.53, 29.45, 29.32, 29.28, 27.23, 27.07, 22.69, 20.75, 14.12; IR (neat) 3438 (OH), 1745 and 1694 (C=O), 1455 cm^{-1}; MS (EI) m/z 435 (M^+). Anal. calcd for $C_{26}H_{45}NO_4$: C, 71.68; H, 10.41; N, 3.22. Found: C, 72.13; H, 10.44; N, 3.19.

{2-(Hydroxymethyl)-4-[(*E*,9*Z*)-9-octadecenylidene]-5-oxo-2-pyrrolidinyl}methyl octanoate (27). This compound was prepared from **23** by following the procedure described for synthesis of **25** in 57% yield as an oil: ^{1}H NMR ($CDCl_3$) δ 6.56 (bs, 1H, NH), 6.50 (m, 1H, >C=CH), 5.35 (m, 2H, CH=CH), 4.22 (d, 1H, J = 11.2 Hz, CO_2CH_2), 4.06 (d, 1H, J = 11.2 Hz, CO_2CH_2), 3.57 (d, 1H, J = 11.5 Hz, CH_2OH), 3.47 (d, 1H, J = 11.5 Hz, CH_2OH), 2.54 (m, 2H, H-4), 2.34 (t, 2H, J = 7.3 Hz, CH_2CO_2), 1.9–2.2 (m, 6H, >C=CHC$\underline{H}_2$ and C$\underline{H}_2$CH=CHC$\underline{H}_2$), 1.2–1.5 (m, 32H), 0.88 (distorted t, 6H); ^{13}C NMR ($CDCl_3$) δ 174.20, 170.51, 136.00, 130.04, 129.76, 125.05, 66.06, 65.58, 59.56, 34.14, 32.62, 31.92, 31.65, 29.78, 29.76, 29.54, 29.47, 29.34, 29.23, 29.11, 28.90, 27.25, 27.20, 24.91, 22.70, 22.61, 14.13, 14.06; IR (neat) 3438 (OH), 1746 and 1665 (C=O), 1456 cm^{-1}; MS (EI) m/z 519 (M^+). Anal. calcd for $C_{32}H_{57}NO_4$: C, 73.94; H, 11.05; N, 2.69. Found: C, 74.21; H, 11.10; N, 2.65.

{5-(Hydroxymethyl)-3-[(*Z*,9*Z*)-9-octadecenylidene]-2-oxotetrahydro-3H-pyrrol-5-yl}methyl octanoate (28). This compound was prepared from **24** by following the procedure described for synthesis of **26** in 58% yield as an oil: ^{1}H NMR ($CDCl_3$) δ 6.15 (bs, 1H, NH), 5.95 (m, 1H, >C=CH), 5.34 (m, 2H, CH=CH), 4.19 (d, 1H, J = 11.5 Hz, CO_2CH_2), 4.05 (d, 1H, J = 11.5 Hz, CO_2CH_2), 3.51 (d, 1H, J = 11.2 Hz, CH_2OH), 3.46 (d, 1H, J = 11.2 Hz, CH_2OH), 2.70 (m, 2H, >C=CHC$\underline{H}_2$), 2.58 (m, 2H, H-4), 2.34 (t, 2H, J = 7.3 Hz, CH_2CO_2), 1.9–2.1 (m, 4H, C$\underline{H}_2$CH=CHC$\underline{H}_2$), 1.2–1.5 (m, 32H), 0.88 (distorted t, 6H); ^{13}C NMR ($CDCl_3$) δ 174.23, 170.68, 140.37, 129.94, 129.87, 127.02, 65.88, 65.42, 59.05, 34.15, 34.09, 31.92, 31.65, 29.80, 29.72, 29.54, 29.46, 29.34, 29.30, 29.11, 28.90, 27.24, 27.08, 24.91, 22.70, 22.61, 14.13, 14.07; IR (neat) 3437 (OH), 1745 and 1664 (C=O), 1455 cm^{-1}; MS (EI) m/z 519 (M^+). Anal. calcd for $C_{32}H_{57}NO_4$: C, 73.94; H, 11.05; N, 2.69. Found: C, 74.23; H, 11.11; N, 2.66.

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