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The Inhibitory Mechanism of Bovine Pancreatic Phospholipase A_2 by Aldehyde Terpenoids

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Abstract: We established the stereoselective synthesis of (E)-3-methoxycarbonyl-2,4,6-trienal compound **A** and discovered that the compound **A** showed more powerful inhibitory activity toward phospholipase A_2 (PLA_2) from bovine pancreas than manoalide which is a typical PLA_2 inhibitor. As the inhibitory mechanism of PLA_2 by **A**, the irreversible formation of dihydropyridine derivative resulting from the reaction of **A** with lysine residues in PLA_2 was proposed based on the model reactions. Furthermore, **A** was found to selectively modify Lys56 which is included in the interfacial recognition site of this enzyme by the MALDI-TOF-MS peptide mapping analyses.

Keywords: Bovine pancreatic phospholipase A_2, (E)-3-Methoxycarbonyl-2,4,6-trienal compounds, Dihydropyridine, Lysine residue

Introduction

Phospholipase A_2 (PLA_2) specifically catalyzes the hydrolysis of the ester linkage at the *sn*-2 position of the important membrane constituents, glycerophospholipids.[1] Normally, unsaturated fatty acids represented by arachidonic acid are stored by an ester linkage at the *sn*-2 position of glycerophospholipids, and the release of arachidonic acid from this position is the rate-limiting step in the production of eicosanoids such as prostaglandins, leukotrienes and thromboxanes through biosynthetic pathways known as the "arachidonic acid cascade".[2] The excess production of the eicosanoids mediates inflammatory responses such as psoriasis, gout, arthritis, and bee stings. For this reason, the compounds which inhibit the hydrolytic ability of PLA_2 have the possibility of being potent anti-inflammatory agents.[3]

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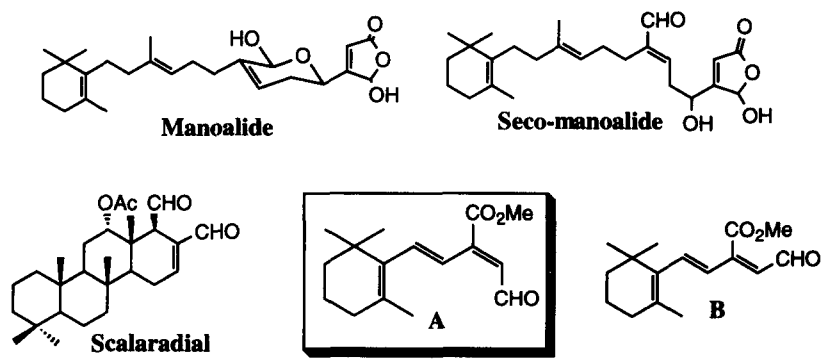
Recently, it has been reported that some unsaturated aldehyde terpenoids inhibit PLA_2.[4] Among these aldehyde terpenoid inhibitors, manoalide[5] and scalaradial,[6] which are sesterterpenoids isolated from marine sponges, are well-characterized and the inhibitory mechanism of manoalide toward PLA_2s isolated mainly from cobra venom and bee venom have been reported.[7, 8] Our group has independently studied to elucidate the inhibitory mechanism of bovine pancreatic PLA_2 by manoalide, [9, 10] since a great deal of information involving the three-dimensional structure and kinetic properties of this enzyme are available.[11]

In our program on the syntheses of new PLA_2 inhibitors and elucidation of their inhibitory mechanisms, we recently discovered that (E)-3-methoxycarbonyl-2,4,6-trienal **A** showed more powerful inhibitory activity than manoalide toward bovine pancreatic PLA_2 and that its (Z)-isomer **B**[12] showed no activity. Our study has focused on understanding the inhibitory mechanism of bovine PLA_2 by compound **A**.

The strategy to elucidate the inhibitory mechanism of bovine pancreatic PLA_2 by compound **A** was based on the following principles. (1) Establishment of an efficient method for the synthesis of compound **A**. (2) Elucidation of the essential functional groups in **A** for inactivation of bovine pancreatic PLA_2. (3) Characterization of the amino acid residue selectively modified by **A** and the elucidation of the irreversible reaction mechanism between the amino acid residue and **A**. In this paper, we disclose the inhibitory mechanisms of PLA_2 by (E)-3-methoxycarbonyl-2, 4, 6-trienal compounds in detail.[13]

Fig.1



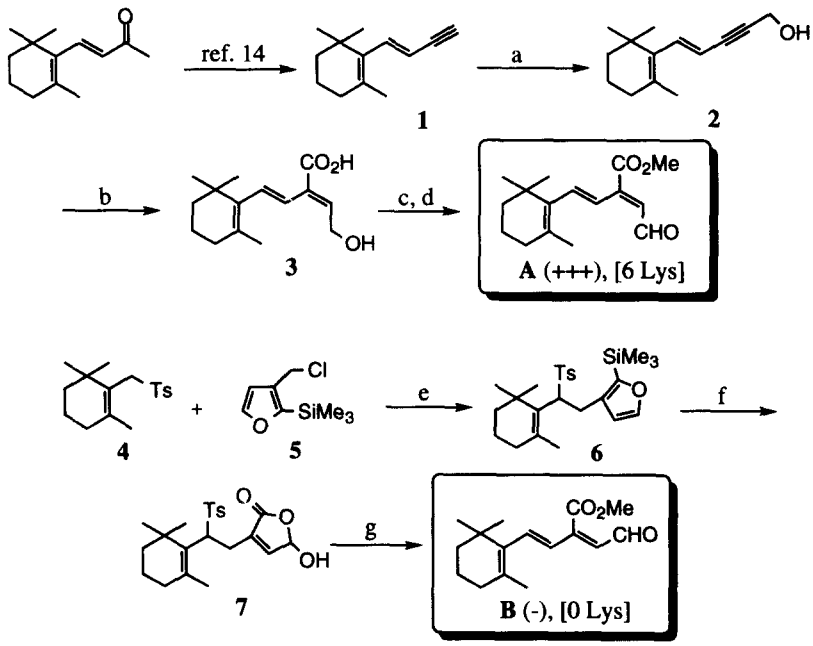
Stereoselective synthesis of compound A.

It was necessary to establish an efficient method for the synthesis of compound **A** , and the method should be applicable to the syntheses of other derivatives in the structure / activity relationship (SAR) study. After several unsuccessful attempts using ionic reactions, the stereoselective synthesis of **A** was finally achieved by hydrometalation on acetylene as the key step (Fig. 2). Thus, an one-carbon elongation of **1**[14] (*n*-butyllithium, *para*-formaldehyde) gave ethynyl alcohol **2** in quantitative yield. As the key step, ethynyl alcohol **2** was treated with excess *iso*-butylmagnesium chloride and a catalytic amount of titanocene dichloride (0.1 equivalent) in ether to produce a vinylmagnesium intermediate, and the solvent was then replaced by tetrahydrofuran and successively reacted with carbon dioxide to give the desired (E)-hydroxylmethyl carboxylic acid **3** in 73% yield.[15, 16] Neither regio nor stereoisomers were detected from the reaction mixtures. Methylesterification was

achieved by treatment of **3** with 1,1,3,3-tetramethylguanidine and methyl iodide in benzene[16] to give the corresponding ester, which was oxidized with manganese dioxide to afford **A** in 71% yield for two steps.

The (Z)-stereoisomer **B**[12] was also stereoselectively synthesized by our own method as follows. The α-lithio derivative of sulfone **4** was alkylated with 2-silyl-3-chloromethylfuran **5** derived from 3-furanmethanol to produce **6** in 86% yield. Then, silylfuran **6** was irradiated with a halogen lamp under an oxygen atmosphere in the presence of tetraphenylporphine to furnish γ-hydroxybutenolide **7** in 91% yield.[17] The stereoselective synthesis of the (Z)-stereoisomer **B** was successfully realized from **7** retaining its Z stereochemistry by treatment of **7** with methyl iodide (12 equivalents) in the presence of diazabicycloundecene (3 equivalents) in dimethylformamide in 82 % yield. The ratio of Z to E stereoisomer was 98 to 2 by NMR analysis.[18] Thus, highly stereoselective syntheses of both (E)- and (Z)-methoxycarbonyltrienal compounds were achieved.

Fig. 2



a) n-BuLi, $(CH_2O)_n$/THF; quant. b) iBuMgCl, Cp_2TiCl_2/ether, then CO_2/THF; 73%
c) N,N,N',N'-tetramethylguanidine, MeI/benzene; 71% d) MnO_2/CH_2Cl_2; quant.
e) n-BuLi/THF; 86% f) $^1O_2/CH_2Cl_2$; 91% g) DBU, MeI/DMF; 82%

Inhibitory activities of compound A and its derivatives toward bovine pancreatic PLA_2.

In the first step toward elucidation of the inhibitory mechanism of PLA_2 by compound **A**, the necessary functional groups in molecule **A** inhibiting bovine pancreatic PLA_2 were examined by SAR study. The various derivatives **C-I** in Fig. 3 were synthesized as followes. Compound **E** was prepared by the above hydromagnesiation method, and the compounds **C**, **D**, and **H** were obtained by photoisomerization of the

corresponding (Z)-stereoisomers, which were yielded by the procedure utilized for the compound **B**. Compound **G** was synthesized from **4** and 3-chloromethyl-5-trimethylsilylfuran. Compound **I** was synthesized by the Wittig reaction of *cis*-dihydrocyclocitral with a new reagent, 3-(2-trimethylsilylfuryl)triphenylphosphonium methylide followed by photosensitized oxygenation, esterification, and isomerization (see experimental section). Then, the inhibitory activities of these synthesized compounds were tested. Bovine pancreatic PLA_2 was reacted with the derivatives **A-I** at appropriate time intervals, and residual PLA_2 activity toward anionic mixed micelles of 1,2-dilauroyl-*sn*-glycero-3-phosphocholine with cholic acid was measured.[9c]

Fig. 3

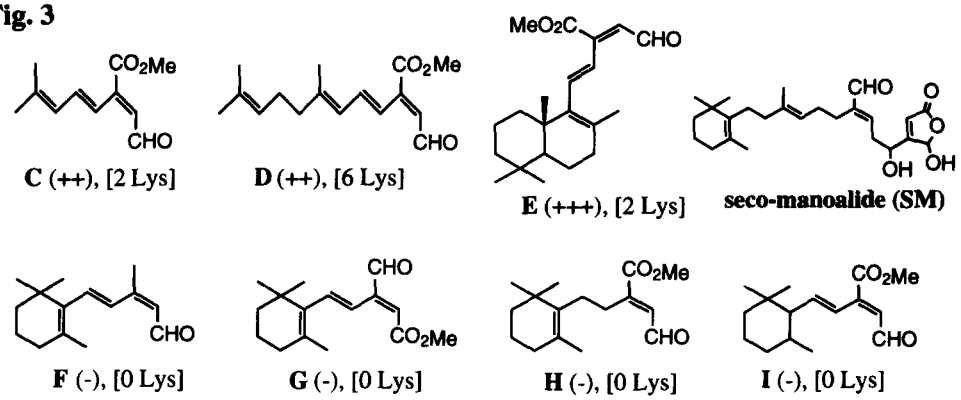
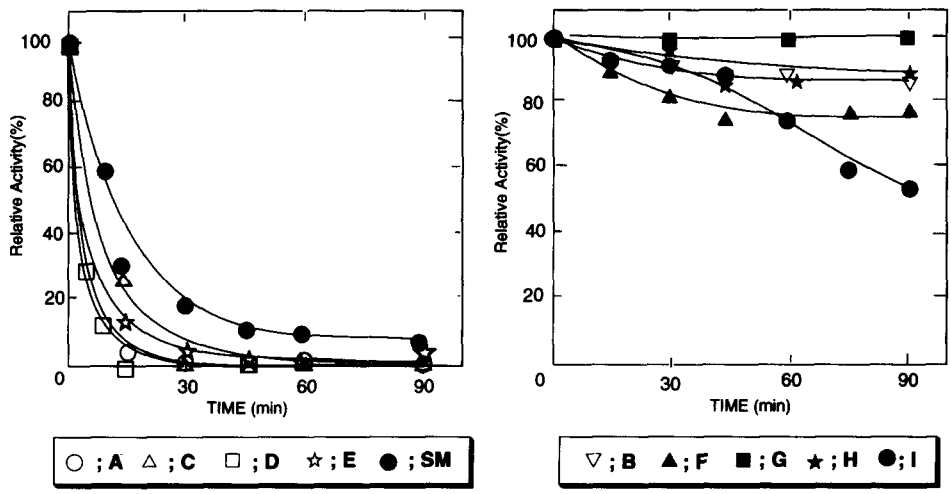


Fig. 4 Inhibition of bovine pancreatic PLA_2 by A and its derivatives.



Bovine pancreatic PLA_2 was incubated with the derivatives **A-I** in 50 mM Tris/HCl buffer at 40 °C and pH 8.0. At appropriate time intervals, PLA_2 activity toward anionic mixed micelles of 1,2-dilauroyl-*sn*-glycero-3-phosphocholine with cholic acid was measured by the pH-statt method at 25 °C, pH 7.0, and ionic strength 0.2 in the presence of 10 mM $CaCl_2$.[9c]

The results are shown in Fig. 4 and summarized in Fig. 3. All (E)-3-methoxycarbonyl-2, 4, 6-trienal compounds **A**, **C**, **D**, and **E** showed powerful inhibitory activities toward PLA_2.[19] On the contrary, no significant inactivation of PLA_2 was observed by the following derivatives, (Z)-stereoisomer **B**, the derivative **F** lacking a methoxycarbonyl group, the derivative **G** in which two carbonyl groups of **A** were exchanged, and the derivatives **H** and **I** lacking the C4 or C6 double bond. It is clear that the (E)-3-methoxycarbonyl-2,4,6-trienal system in a molecule **A** is essential for the inactivation of bovine pancreatic PLA_2. Because the aldehyde group of **A** is essentially required for the PLA_2 inactivation as well as in the case of manoalide, it was predictable that the irreversible reaction of **A** with lysine residues would cause the inactivation of PLA_2.[7,8,9] Based on this assumption, it would be of great interest to identify the amino acid residues irreversibly modified by the derivatives **A**, **C**, **D**, and **E**.

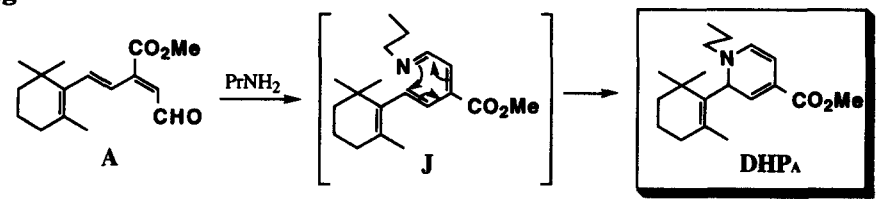
Elucidation of the reaction mechanisms between the (E)-3-methoxycarbonyl-2, 4, 6-trienal compounds and the amino acid residue of PLA_2.

Bovine pancreatic PLA_2 was reacted with the derivatives **A**, **C**, **D**, and **E** for 90 minutes under the same conditions as those of assay, and the reaction derivatives were called the modified PLA_{2A}, PLA_{2C}, PLA_{2D}, and PLA_{2E}, respectively. The composition changes in the amino acid residues in these modified PLA_{2X}s were determined by amino acid analysis. It was found that six out of eleven lysine residues were lost in PLA_{2A} and PLA_{2D} and that two lysine residues were lost in PLA_{2C} and PLA_{2E} (Fig. 2 and 3). None of the other amino acid residues (Leu, Ala, Ile, Arg, etc.) of these modified PLA_{2X}s were lost. Thus, it was elucidated that the derivatives **A**, **C**, **D**, and **E** irreversibly modified only the lysine residues of bovine PLA_2 and inactivated its hydrolytic ability toward anionic micelles of glycerophospholipids by 90-100%. Furthermore, it was found that the derivatives **F-I**, which showed no significant inhibitory activities toward bovine PLA_2, did not modify any amino acid residues of PLA_2. The phenomenon was the same as in the case of manoalide, which also irreversibly reacted with only the lysine residues of bovine PLA_2 to inactivate the enzyme.[9] Our interest was then directed toward investigating how the (E)-3-methoxycarbonyl-2, 4, 6-trienal compounds reacted with the lysine residues of PLA_2 and what irreversible products would be formed by the reaction. We used a primary amine as a model of the lysine residue of PLA_2 and examined the reaction between the (E)-3-methoxycarbonyl-2, 4, 6-trienal compounds and the lysine residues.

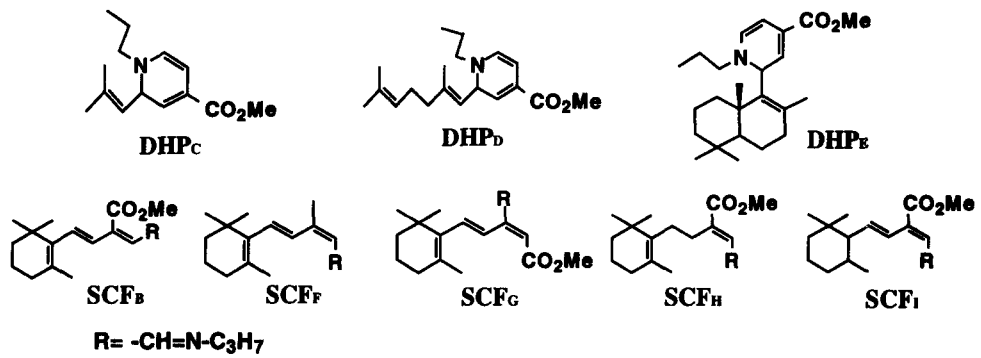
Reaction of **A** with *n*-propylamine in 1, 4-dioxane quantitatively yielded 1, 2-dihydropyridine derivative **DHP**$_A$ within five minutes at room temperature (Fig. 5). The corresponding dihydropyridine derivatives (**DHP**$_C$-**DHP**$_E$) were also obtained from the reaction of the derivatives **C**, **D**, and **E** with *n*-propylamine, respectively.[20] The reaction must proceed via 6π-electrocyclization of the intermediary azatriene **J** as shown in Fig. 5.[21,22] On the other hand, the (Z)-stereoisomer **B** and the derivatives **F**, **G**, **H** and **I**, which showed no significant inhibitory activities toward bovine PLA_2, gave only the corresponding Schiff bases, **SCF**$_B$ and **SCF**$_{F\text{-}I}$, within 60 minutes at room temperature. Obviously, the (E)-3-methoxycarbonyl-2, 4, 6-trienal system in the derivatives **A**, **C**, **D**, and **E** was essential for the smooth 6π-electron electrocyclization of the intermediary 1-azatriene. These required functional groups for rapid cyclization to give the 1, 2-dihydropyridine derivatives are in accordance with those for significant inhibition of PLA_2 by **A**, **C**, **D**, and **E**. Thus, it was strongly suggested that the derivatives **A**, **C**, **D**, and **E** irreversibly reacted with the lysine residues of bovine PLA_2 to give the 1, 2-

dihydropyridine derivatives and then inactivated the enzyme.

Fig. 5



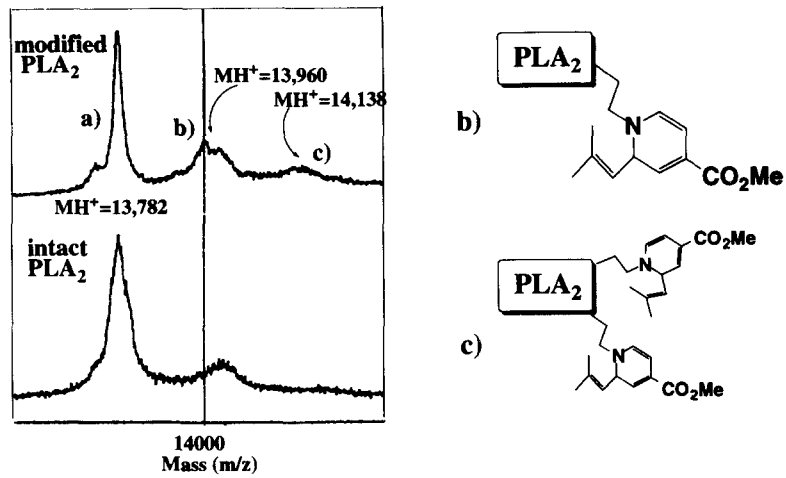
Formation of the dihydropyridine and Schiff base derivatives



Recently, Okamura and coworkers reported that the reaction of 13-*tert*-butyl-13-*cis* retinal with *n*-butylamine for 1 hour at room temperature gave 1,2-dihydropyridine instead of the expected Schiff base. They examined how the steric, electronic, and conformational factors effected the disrotatory 6π-electron electrocyclization through the structure-reactivity studies.[21,22] Our present results are the first observation that both the C3 methoxycarbonyl group and the C6 double bond in (E)-3-methoxycarbonyl-1-aza-2, 4, 6-triene significantly contribute to the acceleration of the aza-6π-electrocyclization reaction.

These model studies were also supported by Matrix Assisted Laser Desorption Ionization Time of Flight (MALDI-TOF) mass spectra of the irreversibly modified PLA_2 by the derivatives **A**, **C**, and **E**. MALDI-TOF-MS has come to be extensively utilized for analysis of proteins and peptides because of its easier operation, capability of providing more accurate information on molecular weights, and wider mass range compared with other techniques of mass spectrometry. In the MALDI-TOF-MS spectra of the samples prepared by treatment of PLA_2 with the derivatives **A**, **C**, and **E**, new ion peaks were found to appear along with the ion peak corresponding to intact PLA_2. For example, the mass spectrum of the PLA_2 prepared by the reaction with **C** for 60 minutes at 40 °C (Fig. 6) showed three peaks, one of which was a molecular weight ion at MH^+=13,960 (theoretical MH^+=13,959) that corresponded to the modified PLA_2 by one molecule of **C** to form a dihydropyridine derivative. The other two peaks appeared at MH^+=14,138 (theoretical MH^+ = 14,135) corresponding to the modified PLA_2 by two molecules of **C** and at MH^+=13,782 (theoretical MH^+=13,783) for the unreacted PLA_2. Similarly, the mass spectra showed the ion peaks which corresponded to the modified PLA_2 by **A** and **E** to form dihydropyridine derivatives. Thus, the results of the model reactions between *n*-propylamine and the derivatives **A**, **C**, **D**, and **E** strongly supported the inactivation mechanism of PLA_2 by these trienal compounds.

Fig. 6 MALDI - TOF MASS spectrum of the modified PLA_2 by the derivative C.



Identification of the lysine residues irreversibly modified by the (E)-3-methoxycarbonyl-2, 4, 6-trienal compounds.

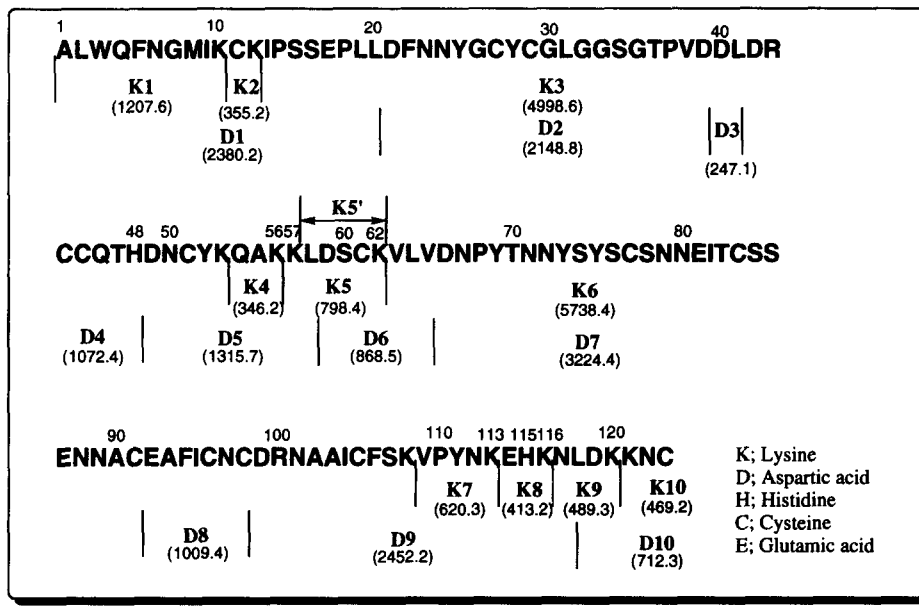
Subsequently, identification of the lysine residues in bovine PLA_2, which were modified by (E)-3-methoxycarbonyl-2, 4, 6-trienal compounds, was attempted. Thus, both intact PLA_2 and the irreversibly modified PLA_2 by the derivative **E** were subjected to enzyme digestion after S-S bonds cleavages by dithiothreitol treatment and subsequent protection of the resulting -SH groups with 4-vinylpyridine treatment.[9c,23] The structures of the fragments were then analyzed using the MALDI-TOF-MS peptide mapping method. Lysyl endopeptidase (Lys-C) selectively cleaves the C-terminal bond of lysine residues, and Asp-N selectively cleaves the N-terminal bond of aspartic acid residues.

The amino acid sequence of bovine pancreatic PLA_2 is shown in Fig. 7, and the MALDI-TOF-MS Lys-C digest maps of both intact PLA_2 and the modified PLA_2 by the derivative **E** are shown in Fig. 8. The digestion of intact PLA_2 by Lys-C gave the peptide fragments K1 to K10 as shown in Fig. 7. When the Lys-C digest map of modified PLA_2 by the derivative **E** is compared with that of intact PLA_2, four new fragments O, P, Q, and R are found in the former (Fig. 8). These new fragments are identified as follows. The fragment O corresponds to the peptide fragment K5' in Fig. 7. The appearance of the fragment K5' evidently indicated that Lys56 was modified by the derivative **E** based on the following analysis. When a peptide has a continuous sequence of lysine residues such as X-Lys-Lys-Y, Lys-C generally cleaves the peptide bond between the two neighboring Lys groups preferentially to the bond between Lys and Y. Because bovine pancreatic PLA_2 contains lysine residues at positions 56 and 57, the peptide bond between Lys56 and Lys57 is generally cleaved by Lys-C to give the peptide fragments K4 and K5. However, when Lys56 was irreversibly modified by derivative **E**, the peptide bond between Lys56 and Lys57 was not cleaved, but the C-terminal bonds of Lys57 and Lys62 were cleaved to give the fragment K5'. Thus, the modification of Lys56 was apparently elucidated.

The fragment P corresponds to the peptide fragment which contains the Lys56 irreversibly modified by the

derivative **E**. When the derivative **E** reacted with Lys56 and the dihydropyridine derivative of **E** was formed, the peptide fragment K4 plus Lys57 plus the dihydropyridine derivative would be found at MH^+=786.8 (Fig. 7). However, the molecular weight of the observed fragment P (MH^+=770.7) is 16 mass units lower than that of the theoretical fragment. The fragment Q originates from the modification of Lys116 by the derivative **E**. When Lys116 was modified by the derivative **E**, the peptide bond between K8 and K9 would not be cleaved, and the new peptide fragment consisting of K8 plus K9 plus the dihydropyridine derivative would be found at MH^+=1195.9. The molecular weight of the observed fragment Q (MH^+=1179.8) is also 16 mass units lower than the theoretical value. The fragment R originates from the modification of Lys113 by the derivative **E**. Similarly, when Lys113 was modified by the derivative **E**, the new peptide fragment K7 plus K8 plus the dihydropyridine derivative would be found at MH^+=1327.0. However, the molecular weight of the fragment R (MH^+=1310.8) is also 16 mass units lower than that of the theoretical fragment.

Fig. 7 Amino acid sequence of bovine pancreatic PLA_2



K; Lys-C (lysyl endopeptidase) digestion
D; Asp-N digestion
( ); theoretical molecular weight; MH^+

The CID MS/MS (Collision Induced Dissociation Mass Spectrometry-Mass Spectrometry) technique also elucidated the structures of both the fragments Q and R. Thus, the CID MS/MS analysis of these fragments detected the ion peak at MH^+=110 which was due to the characteristic histidine immonium ion, and therefore, both of the fragments Q and R include a histidine residue. Bovine pancreatic PLA_2 contains only two histidine residues, one of which is at position 115, and it is included in the peptide fragment K8 (Fig. 7). This strongly supports the belief that each fragment Q and R must be derived from the peptide fragment K8 plus K9 plus the dihydropyridine derivative of **E**, and K7 plus K8 plus the dihydropyridine derivative, respectively. Thus, the

Fig. 8 MALDI-TOF mass spectra of digests by Lys-C.

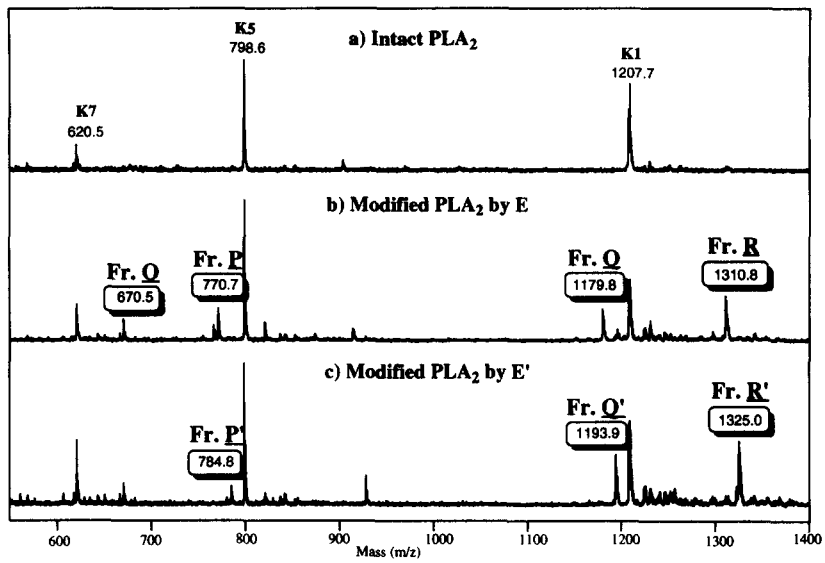
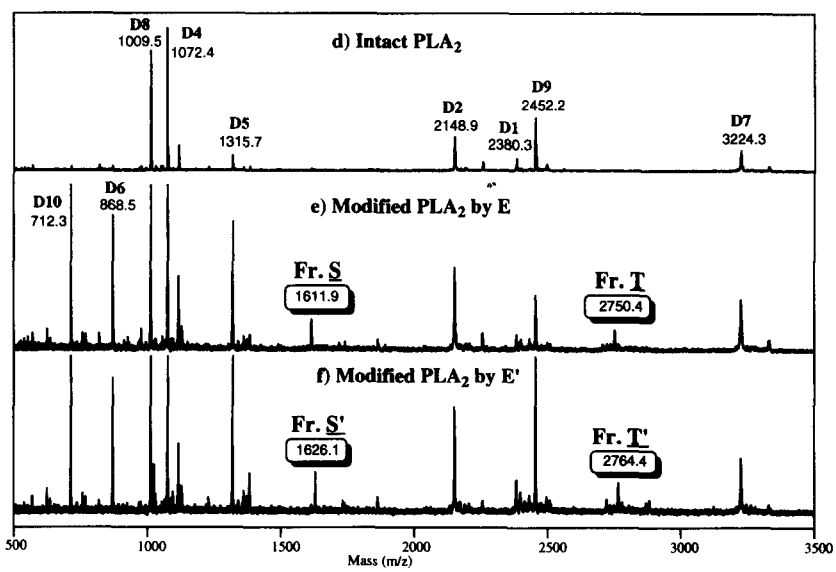
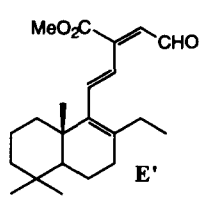


Fig. 9 MALDI-TOF mass spectra of digests by Asp-N.



above results from Lys-C digest maps are certainly understandable, if the derivative **E** was assumed to modify Lys56, Lys113, and Lys116, respectively. However, the fragments P-R, which would contain the irreversibly modified lysine residues by the derivative **E**, showed 16 mass unit lower molecular weights than those of the theoretical fragments.

Subsequently, intact and modified PLA_2s were digested by Asp-N. The digestion of intact PLA_2 by Asp-N gave the peptide fragments D1 to D10 (Fig. 7). In the digest map of modified PLA_2, the new fragments S and T are observed by comparison with that of intact PLA_2 (Fig. 9). The fragment S corresponds to the peptide fragment D5 plus the dihydropyridine derivative of **E**, which was generated from the modification of Lys56. However, the molecular weight of the observed fragment S at MH^+=1611.9 is 16 mass units lower than that of the theoretical fragment (MH^+=1628.2). The fragment T corresponds to the peptide fragment D9 plus the dihydropyridine derivative of **E**, which was generated from the modification of Lys113 or Lys116. Similarly, the molecular weight of the fragment T at MH^+=2750.4 is 16 mass units lower than the theoretical value (MH^+=2766.3). Thus, all of the fragments P-T showed 16 mass unit lower molecular weights than those of the expected peptide fragments, which were generated from the irreversible modification of Lys56, Lys113, and Lys116 by the derivative **E** to form dihydropyridine derivatives. It is possible that 1, 2-dihydropyridine derivative would decompose into an unidentified substance, whose molecular weight would be 16 mass units lower than that of the dihydropyridine derivative.[24]



The above considerations concerning the results obtained from the digest maps are based on the assumption that the derivative **E** would irreversibly modify the lysine residues in the fragments P-T. To prove this assumption, the following experiment was conducted. We decided to use the derivative **E'**, which has an ethyl group instead of the C-8 methyl group of the derivative **E**, and was prepared from 2-diethoxyphosphoxy-1-methoxycarbonyl-5,5,8a-trimethyloctahydronaphthalene[25] by the introduction of an ethyl group followed by the same procedure as the case of the compound **D** (see experimental section). The PLA_2 modified by the derivative **E'** was digested by both Lys-C and Asp-N. The molecular weight of the derivative **E'** is 14 mass units different from that of the derivative **E**, and this difference would be clearly detectable as the fragments P'-T' which would also have 14 mass unit larger molecular weights than those of the fragments P-T, if the above assumption is appropriate. The Lys-C digest map of the PLA_2 modified by **E'** showed the new fragments P', Q', and R' (Fig. 8, spectrum c). They respectively have 14 mass unit larger molecular weights than those of the corresponding fragments P, Q, and R, which were observed in the digest map of the PLA_2 modified by **E**. Similarly, the Asp-N digest map of the PLA_2 modified by **E'** showed the fragments S' and T' as new fragments (Fig. 9, spectrum f). They also have 14 larger mass units than the fragments S and T in the digest map of PLA_2 modified by **E**. Thus, it was proved that the fragments P-T included the irreversibly modified lysine residues by the derivative **E** or **E'**, and the modifications of Lys56, Lys113, and Lys116 were then concluded.

On the hydrolytic mechanism of glycerophospholipids by PLA_2, the presence of the interfacial recognition site for micellar phospholipids in addition to the catalytic site, which consists of His48 and Asp99, has been accepted based on the fact that micellar phospholipids are hydrolyzed ten thousand times faster than unimolecular dispersed phospholipids. Noel and coworkers reported based on their experimental results utilyzing site-directed mutagenesis of bovine pancreatic PLA_2 that Lys56 would be included in the interfacial recognition site of this enzyme, where interfacial bindings between PLA_2 and anionic micelles of glycerophospholipids take

place.[26] Therefore, it can be concluded that the 1, 2-dihydropyridine derivative formed by the reaction of Lys56 with (E)-3-methoxycarbonyl-2, 4, 6-trienal compounds would disturb the interfacial bindings between PLA_2 and anionic micelles of the substrates, which resulted in the inactivation of the enzyme.

Conclusions

The obtained results of the inhibitory mechanism of bovine pancreatic PLA_2 by compound **A** are summarized in the following three points. (1) The methoxycarbonyl group at the 3 position and the conjugated trienal system in molecule **A** were essential for the inactivation of bovine pancreatic PLA_2, and little effect of the hydrophobic part of **A** on the inhibitory activity was observed. (2) The model studies suggested that the (E)-3-methoxycarbonyl-2, 4, 6-trienal compounds **A**, **C**, **D** and **E** irreversibly reacted with lysine residues of bovine pancreatic PLA_2 to yield 1, 2-dihydropyridine derivatives *via* aza-6π-electrocyclization of intermediary Schiff bases and inactivated this enzyme. This conclusion obtained from the model studies was strongly supported by the MALDI-TOF mass spectra of the PLA_2s irreversibly modified by **A**, **C**, **D**, and **E**. (3) Based on the MALDI-TOF-MS peptide mapping analyses, the derivative **E** was found to irreversibly and selectively react with Lys56, Lys113, and Lys116 out of eleven lysine residues in bovine PLA_2. The irreversible 1, 2-dihydropyridine derivative would eliminate the positive charge at Lys56 in the interfacial recognition site of this enzyme and would disturb the interfacial bindings between the anionic micelles of glycerophospholipids and PLA_2, which resulted in the decrease in the enzymic activity.

Now, we have presently known the three aldehyde terpenoids, scalaradial, manoalide, and (E)-3-methoxycarbonyl-2, 4, 6-trienal compound **A**, which strongly inactivate PLA_2. Scalaradial also irreversibly reacts with lysine residues to inactivate PLA_2, and the production of the pyrrole derivative has been proposed by Cimino and coworkers based on the model reaction with methylamine.[6b] In the case of manoalide, our group has found that the irreversible modification of Lys56 in the interfacial recognition site of bovine PLA_2 by manoalide is responsible for the inactivation of this enzyme.[9] However, a clear answer about how the irreversible reaction proceeded between manoalide and the lysine residues of PLA_2, was not obtained. Although the real reaction mechanism has not been known, our model studies on the reactions between manoalide analogs and mono- and diamines strongly suggested that manoalide reacted with the two close lysine residues to give the polymerized compounds.[10] Moreover, in the present case of the (E)-3-methoxycarbonyl-2, 4, 6-trienal compound **A**, the inactivation mechanism was summarized previously.

Thus, it would be concluded that the formation of the irreversible product by the reaction with the lysine residue in interfacial recognition site of PLA_2 is essential for sufficient inactivation of PLA_2 by unsaturated aldehyde terpenoids such as manoalide, scalaradial, and the (E)-3-methoxycarbonyl-2, 4, 6-trienal compounds.

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Experimental section

All commercially available reagents were used without further purification. All solvents were used after

distillation. Tetrahydrofuran and diethylether were refluxed over and distilled from sodium. Dichloromethane and diisopropylamine were refluxed over and distilled from CaH_2. Dimethylformamide (DMF) and dimethyl sulfoxide (DMSO) were distilled from CaH_2 under reduced pressure. Methanol and ethanol were refluxed over and distilled from magnesium. Preparative separation was usually performed by column chromatography on silica gel (FUJI silysia LTD, BW-200 and BW-300). ^1H NMR and ^{13}C NMR spectra were recorded on a JEOL α-400 spectrometer and chemical shifts were represented as δ-values relative to the internal standard TMS. IR spectra were recorded on a Hitachi 270-30 spectrometer. High resolution mass spectra (HRMS) were measured on a JEOL JMS-HX110A / 110A Tandem Mass Spectrometer.

(E)-5-(2,6,6-Trimethylcyclohex-1-enyl)pent-4-en-2-yn-1-ol (2). To a THF (35 mL) solution of **1** (2.0 g, 11.5 mmol) was added dropwise *n*-butyllithium (1.6 M solution in hexane, 7.89 mL, 12.6 mmol) at -55 °C over 20 min. The reaction mixture was stirred at -55 °C for 30 min and paraformaldehyde (1.73 g, 57.5 mmol) was added at this temperature. After the mixture was warmed to room temperature and stirred for an additional 1 h, saturated aqueous NH_4Cl solution was added, and the resulting mixture was extracted with ether. The organic layers were combined, washed with saturated $NaHCO_3$ solution, brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* to give the crude products. Column chromatography on silica gel (from 2 % to 9 % ether in hexane) gave alcohol **2** (2.34 g, 100 %): IR (KBr disk, cm^{-1}) 3284 (br), 2212, 1462, 1362, 1170, 1132, 628; ^1H NMR (400 MHz, $CDCl_3$) δ 1.01 (s, 6H), 1.43-1.46 (m, 2H), 1.56-1.69 (m, 2H), 1.71 (s, 3H), 2.00 (t, 2H, J = 6.0 Hz), 4.42 (m, 2H), 5.48 (dt, 1H, J = 16.4, 2.0 Hz), 6.60 (d, 1H, J = 16.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.03, 21.51, 28.70, 33.03, 33.97, 39.46, 51.69, 85.30, 87.07, 111.20, 131.63, 136.84, 141.67; FAB HRMS m/z calcd. for $C_{14}H_{20}O$ (M^+) 204.1514, found 204.1519.

(E,E)-4-Hydroxy-2-[(2,6,6-trimethylcyclohex-1-enyl)vinyl]but-2-enoic acid (3). To a 0.88 M solution of *iso*butylmagnesium chloride in ether (20 mL, 17.6 mmol) prepared from *iso*butyl chloride (3 mL, 28.5 mmol), magnesium turnings (727 mg, 29.9 mmol), and ether (19 mL) was added titanocene dichloride (82 mg, 0.33 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 20 min and a ether (10 mL) solution of **2** (450 mg, 2.2 mmol) was added dropwise at the same temperature over 10 min. The mixture was warmed to room temperature and stirred for an additional 6 h. The bulk of ether was removed *in vacuo*, and THF (12 mL) was added. After stirring at 0 °C for 12 h under an atmosphere of carbon dioxide, 2 N HCl solution was added, and the resulting mixture was extracted with ethyl acetate. The organic layers were combined, washed with 2 N HCl solution, brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* to give the crude products. Column chromatography on silica gel (from 17 % to 50 % ethyl acetate in hexane) gave acid **3** (401 mg, 73 %): IR (KBr disk, cm^{-1}) 3300 (br), 2936, 1694, 1632, 1418, 1364, 1262, 1048; ^1H NMR (400 MHz, $CDCl_3$) δ 1.02 (s, 6H), 1.45-1.48 (m, 2H), 1.58-1.64 (m, 2H), 1.73 (s, 3H), 2.01 (t, 2H, J = 6.0 Hz), 4.54 (d, 2H, J = 6.0 Hz), 6.09 (d, 1H, J = 16.3 Hz), 6.22 (d, 1H, J = 16.6 Hz), 6.93 (t, 1H, J = 6.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.14, 21.68, 28.81, 32.89, 34.01, 39.39, 60,27. 124.72, 130.42, 130.63, 135.12,, 137.45, 141.67, 172.02; FAB HRMS m/z calcd. for $C_{15}H_{22}O_3$ (M^+) 250.1569 found 250.1581.

Methyl (E,E)-4-hydroxy-2-[(2,6,6-trimethylcyclohex-1-enyl)vinyl]but-2-enoate. To a solution of **3** (100 mg, 0.40 mmol) in benzene (3 mL) was added N,N,N',N'-tetramethylguanidine (0.1 mL, 0.8 mmol) at room temperature. The reaction mixture was stirred for 1 h and methyl iodide (0.25 mL, 4.0 mmol) was added

at the same temperature. After stirring at room temperature for 12 h, the reaction mixture was filtered and concentrated *in vacuo* to give the crude products which were purified by column chromatography on silica gel (2 % ethyl acetate in hexane) to afford the corresponding methyl ester (70 mg, 71 %): IR (neat, cm^{-1}) 1628, 1362, 1062, 800, 744, 668; ^1H NMR (400 MHz, $CDCl_3$) δ 1.02 (s, 6H), 1.45-1.48 (m, 2H), 1.59-1.73 (m, 2H), 1.73 (s, 3H), 2.01 (t, 2H, J = 6.4 Hz), 3.78 (s, 3H), 4.50 (d, 2H, J = 6.0 Hz), 6.08 (d, 1H, J = 16.4 Hz), 6.22 (d, 1H, J = 16.4 Hz), 6.77 (t, 1H, J = 6.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.13, 21.63, 28.81, 32.88, 34.01, 39.38, 51.98, 60.09, 125.03, 130.24, 131.33, 134.66, 137.49, 139.49, 167.55.

Methyl (E,E)-4-oxo-2-[(2,6,6-trimethylcyclohex-1-enyl)vinyl]but-2-enoate (A).[12] To a solution of the methyl ester obtained above (65 mg, 0.246 mmol) in dichloromethane (5 mL) was added manganese dioxide (1.4 g) at room temperature, and the mixture was stirred for 6 min. The reaction mixture was filtered and concentrated *in vacuo* to afford **A** (64 mg, 100 %), whose spectral data were in good agreement with those reported[12]: IR (neat, cm^{-1}) 1738, 1730, 1678, 1582, 1442, 1246, 1142; ^1H NMR (400 MHz, $CDCl_3$) δ 1.07 (s, 6H), 1.47-1.50 (m, 2H), 1.60-1.66 (m, 2H), 1.79 (s, 3H), 2.07 (t, 2H, J = 6.4 Hz), 3.87 (s, 3H), 6.63 (d, 1H, J = 7.2 Hz), 6.64 (s, 2H), 10.05 (d, 1H, J = 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.93, 21.85, 28.87, 33.31, 34.11, 39.49, 52.74, 123.31, 131.20, 134.43, 137.27, 141.81, 146.76, 167.17, 191.48.

3-[2,2-(2,6,6-Trimethylcyclohex-1-enyl)-*p*-toluenesulfonylethyl]-2-trimethylsilylfuran (6).[17] To a THF (30 mL) solution of cyclocitryl sulfone **4** (1.39 g, 4.76 mmol) was added dropwise *n*-butyllithium (1.6 M solution in hexane, 3.57 mL, 5.71 mmol) at -78 °C. The reaction mixture was stirred at -78 °C for 20 min and a THF (2.0 mL) solution of 2-silyl-3-chloromethylfuran **5**[17] (1.17 g, 6.19 mmol) was added to this solution at the same temperature. After the mixture was gradually warmed to room temperature and stirred for an additional 12 h, H_2O was added, and the resulting mixture was extracted with ether. The organic layers were combined, washed with H_2O, brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* to give the crude products. Column chromatography on silica gel (1 % ethyl acetate in hexane) gave furan derivative **6** (2.03 g, 96 %): IR (nujol, cm^{-1}) 1598, 1468, 1314, 1304, 1252, 1144, 1088, 842; ^1H NMR (400 MHz, $CDCl_3$) δ 0.14 (s, 9H), 0.44 (s, 3H), 1.06 (s, 3H), 1.34-1.39 (m, 2H), 1.49-1.53 (m, 2H), 2.01-2.23 (m, 2H), 2.11 (s, 3H), 2.44 (s, 3H), 3.16 (dd, 1H, J = 8.4, 14.9 Hz), 3.35 (dd, 1H, J = 5.7, 14.9 Hz), 3.87 (dd, 1H, J = 8.4, 5.7 Hz), 5.68 (d, 1H, J = 1.5 Hz), 7.31 (d, 2H, J = 8.3 Hz), 7.35 (d, 1H, J = 1.5 Hz), 7.73 (d, 2H, J = 8.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ -1.31, 18.97, 21.55, 23.28, 26.67, 27.14, 29.30, 34.62, 35.78, 39.57, 68.42, 111.12, 128.38, 129.62, 130.04, 131.39, 138.00, 138.98, 143.94, 146.10, 155.46.

4-Hydroxy-2-[2,2-(2,6,6-trimethylcyclohex-1-enyl)-*p*-toluenesulfonylethyl]butenolide (7).[17] To a solution of **6** (2.0 g, 4.50 mmol) in dichloromethane (30 mL) was added a catalytic amount of tetraphenylporphine (TPP), and the solution was cooled at -78 °C. The mixture was irradiated with a halogen lamp (Riko-HGA-300A) at -78 °C under an oxygen atmosphere, and the reaction was monitored by TLC. After the starting material was consumed, the reaction mixture was flushed with argon. The solvent was removed *in vacuo* and the crude products were purified by column chromatography on silica gel (from 2 % to 50 % ethyl acetate in hexane) to give γ-hydroxybutenolide **7** (1.64 g, 91 %) as a mixture of stereoisomers: IR (nujol, cm^{-1}); 3440, 1766, 1752, 1598, 1460, 1300, 1142; ^1H NMR (400 MHz, $CDCl_3$) δ 0.75 (s, 1/2×3H), 0.90 (s, 1/2×3H), 0.92 (s, 1/2×3H), 0.96 (s, 1/2×3H), 1.35-1.55 (m, 4H), 1.97-2.11 (m, 2H), 2.02 (s, 1/2×3H), 2.05 (s,

1/2×3H), 2.43 (s, 3H), 2.76-2.83 (m, 1H), 3.37-3.49 (m, 1H), 4.31-4.38 (m, 1H), 4.50 (brs, 1H), 5.94 (brd, 1/2×1H, J = 6.8 Hz), 5.98 (brd, 1/2×1H, J = 6.1 Hz), 6.88 (s, 1/2×1H), 6.97 (s, 1/2×1H), 7.31 (d, 2H, J = 8.1 Hz), 7.66-7.70 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.83, 18.88, 21.60, 23.14, 23.17, 27.49, 27.74, 28.18, 28.22, 28.35, 28.63, 34.61, 34.68, 36.23, 36.32, 39.59, 39.61, 64.26, 65.24, 96.61, 96.84, 128.44, 128.72, 129.58, 130.67, 130.72, 134.00, 134.08, 137.52, 138.28, 138.33, 144.53, 144.69, 146.43, 146.56, 170.70, 170.88.

Methyl (Z,E)-4-oxo-2-[(2,6,6-trimethylcyclohex-1-enyl)vinyl]but-2-enoate (B).[12] To a solution of **7** (1.46 g, 3.72 mmol) in DMF (20 mL) was added diazabicycloundecene (1.7 mL, 11.2 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 6 h and methyl iodide (2.8 mL, 44.7 mmol) was added at the same temperature. After the mixture was stirred at room temperature for 1 h, saturated aqueous NH_4Cl solution was added, and the resulting mixture was extracted with ether. The organic layers were combined, washed with saturated $NaHCO_3$ solution, brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* to give the crude products. Column chromatography on silica gel (4 % ethyl acetate in hexane) gave **B** (798 mg, 82 %, E : Z = 2 : 98), whose spectral data were in good agreement with those reported[12]: IR (neat, cm^{-1}) 1772, 1736, 1680, 1582, 1442, 1246, 1142; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.05 (s, 6H), 1.46-1.49 (m, 2H), 1.59-1.65 (m, 2H), 1.76 (s, 3H), 2.06 (t, 2H, J = 6.2 Hz), 3.94 (d, 3H, J = 0.7 Hz), 6.07 (d, 1H, J = 7.6 Hz), 6.22 (d, 1H, J = 16.3 Hz), 6.69 (d, 1H, J = 16.3 Hz), 9.76 (dd, 1H, J = 0.7, 7.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.88, 21.76, 28.84, 33.44, 34.22, 39.54, 52.59, 127.64, 128.15, 135.13, 136.84, 139.76, 150.53, 166.77, 190.72.

3-[2,2-(2-Methyl-1-propenyl)-*p*-toluenesulfonylethyl]-2-trimethylsilylfuran. To a THF (70 mL) solution of prenyl sulfone (3.25 g, 14.5 mmol) was added dropwise *n*-butyllithium (1.6 M solution in hexane, 9.95 mL, 15.9 mmol) at -78 °C. The reaction mixture was stirred at -78 °C for 30 min and a THF (10 mL) solution of chloride **5** (3.0 g, 15.9 mmol) was added at the same temperature. After the mixture was gradually warmed to room temperature and stirred for an additional 12 h, H_2O was added, and the resulting mixture was extracted with ether. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* to give the crude products. Column chromatography on silica gel (1 % ethyl acetate in hexane) gave the corresponding furan derivative (4.15 g, 76 %): IR (neat, cm^{-1}); 1598, 1446, 1300, 1288, 1246, 1132, 1088, 844; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.25 (s, 9H), 1.06 (d, 3H, J = 1.5 Hz), 1.63 (d, 3H, J = 1.5 Hz), 2.44 (s, 3H), 2.76 (dd, 1H, J = 14.2, 11.2 Hz), 3.38 (dd, 1H, J = 14.2, 2.9 Hz), 3.81 (ddd, 1H, J = 11.2, 10.3, 2.9 Hz), 5.01 (dd, 1H, J = 10.3, 1.5 Hz), 6.14 (d, 1H, J = 1.7 Hz), 7.32 (d, 2H, J = 8.1 Hz), 7.46 (d, 1H, J = 1.7 Hz), 7.73 (d, 2H, J = 8.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ -1.09, 17.86, 21.58, 24.26, 25.75, 65.89, 111.10, 116.97, 129.13, 129.38, 130.36, 134.86, 142.12, 144.38, 146.08, 155.77; FAB HRMS m/z calcd. for $C_{20}H_{29}O_3SiS$ $(M+H)^+$ 377.1607, found 377.1604.

4-Hydroxy-2-[2,2-(2-methyl-1-prenyl)-*p*-toluenesulfonylethyl]butenolide. To a solution of the furan derivative obtained above (1.5 g, 3.98 mmol) in dichloromethane (30 mL) was added a catalytic amount of tetraphenylporphine (TPP), and the solution was cooled at -78 °C. The mixture was irradiated with a halogen lamp (Riko-HGA-300A) at -78 °C under an oxygen atmosphere, and the reaction was monitored by TLC. After the starting material was consumed, the reaction mixture was flushed with argon. The solvent was removed *in*

vacuo and the crude products were purified by column chromatography on silica gel (from 2 % to 50 % ethyl acetate in hexane) to give the corresponding γ-hydroxybutenolide (1.05 g, 79 %) as a mixture of stereoisomers: IR (nujol, cm^{-1}); 3368, 1764, 1596, 1446, 1284, 1140; ^1H NMR (400 MHz, $CDCl_3$) δ 1.16 (s, 1/2×3H), 1.22 (s, 1/2×3H), 1.64 (s, 1/2×3H), 1.65 (s, 1/2×3H), 2.45 (s, 3H), 2.61-2.68 (m, 1H), 3.06-3.11 (m, 1H), 4.24-4.29 (m, 1H), 4.88 (brs, 1/2×1H), 4.90 (brs, 1/2×1H), 4.99 (brs, 1H), 6.07 (brs, 1H), 6.88 (brs, 1/2×1H), 6.94 (brs, 1/2×1H), 7.33 (d, 2H, J = 8.3 Hz), 7.69 (d, 2H, J = 8.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.04, 21.62, 24.47, 24.65, 25.71, 61.66, 62.02, 96.98, 115.89, 129.14, 129.53, 133.13, 133.35, 134.02, 143.57, 144.90, 145.94, 146.39, 171.04; FAB HRMS m/z calcd. for $C_{17}H_{21}O_5S$ $(M+H)^+$ 337.1110, found 337.1135.

Methyl (E,E)-4-oxo-2-[(2-methyl-1-propenyl)vinyl]but-2-enoate (C) and methyl (Z,E)-4-oxo-2-[(2-methyl-1-propenyl)vinyl]but-2-enoate. To a solution of the γ-hydroxybutenolide obtained above (300 mg, 0.892 mmol) in DMF (9.0 mL) was added diazabicycloundecene (0.40 mL, 2.68 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 2.5 h and methyl iodide (0.67 mL, 10.7 mmol) was added at the same temperature. After the mixture was stirred at room temperature for 1 h, saturated aqueous NH_4Cl solution was added, and the resulting mixture was extracted with ether. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* to give the crude products. Column chromatography on silica gel (5 % ethyl acetate in hexane) gave 1 : 10 mixture of **C** and its (Z)-stereoisomer (136 mg, 79 %). These compounds were separated by column chromatography on silica gel (1 % ethyl acetate in hexane): Data for (Z)-stereoisomer: IR (neat, cm^{-1}); 1732, 1670, 1590, 1440, 1246, 1142; ^1H NMR (400 MHz, $CDCl_3$) δ 1.85 (s, 3H), 1.89 (s, 3H), 3.95 (s, 3H), 6.00 (d, 1H, J = 11.2 Hz), 6.07 (d, 1H, J = 7.8 Hz), 6.20 (d, 1H, J = 15.4 Hz), 6.90 (dd, 1H, J = 11.2, 15.4 Hz), 9.75 (d, 1H, J = 7.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.03, 26.60, 52.57, 125.18, 125.24, 127.64, 136.39, 145.19, 150.31, 166.64, 190.54. Data for (E)-stereoisomer **C**: IR (neat, cm^{-1}); 1720, 1678, 1634, 1596, 1440, 1134; ^1H NMR (400 MHz, $CDCl_3$) δ 1.86 (s, 3H), 1.89 (s, 3H), 3.87 (s, 3H), 6.06 (d, 1H, J = 11.2 Hz), 6.50 (d, 1H, J = 7.3 Hz), 6.76 (d, 1H, J = 15.1 Hz), 7.04 (dd, 1H, J = 11.2, 15.1 Hz), 10.09 (d, 1H, J = 7.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.01, 26.53, 52.67, 119.77, 125.47, 129.70, 138.74, 144.63, 146.23, 167.09, 191.18; FAB HRMS m/z calcd. for $C_{11}H_{15}O_3$ $(M+H)^+$ 195.1021, found 195.1007.

3-[2,2-(2,6-Dimethyl-1,5-heptadienyl)-*p*-toluenesulfonylethyl]-2-trimethylsilylfuran. To a THF (60 mL) solution of geranyl sulfone (2.49 g, 8.50 mmol) was added dropwise *n*-butyllithium (1.6 M solution in hexane, 5.85 mL, 9.35 mmol) at -78 °C. The reaction mixture was stirred at -78 °C for 30 min and a THF (10 mL) solution of chloride **5** (1.76 g, 9.35 mmol) was added at the same temperature. After the mixture was gradually warmed to room temperature and stirred for an additional 12 h, H_2O was added, and the resulting mixture was extracted with ether. The organic layers were combined, washed with H_2O, brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* to give the crude products. Column chromatography on silica gel (1 % ethyl acetate in hexane) gave the corresponding furan derivative (2.94 g, 78 %): IR (neat, cm^{-1}); 1598, 1450, 1314, 1304, 1250, 1148, 1086, 842; ^1H NMR (400 MHz, $CDCl_3$) δ 0.26 (s, 9H), 1.07 (d, 3H, J = 1.2 Hz), 1.57 (s, 3H), 1.67 (s, 3H), 1.91 (brs, 4H), 2.44 (s, 3H), 2.76 (dd, 1H, J = 13.9, 11.2 Hz), 3.42 (dd, 1H J = 13.9, 2.9 Hz), 3.85 (ddd, 1H, J = 11.2, 11.2, 2.9 Hz), 4.96 (dd, 1H, J = 1.3 Hz), 5.01 (d, 1H, J = 10.2

Hz), 6.15 (d, 1H, J = 1.5 Hz), 7.31 (d, 2H, J = 8.3 Hz), 7.46 (d, 1H, J = 1.5 Hz), 7.74 (d, 2H, J = 8.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ -1.05, 16.32, 17.62, 21.61, 24.24, 25.62, 26.06, 39.68, 65.71, 111.18, 116.89, 123.53, 129.23, 129.37, 130.40, 131.90, 134.89, 144.39, 145.61, 146.05, 155.77; FAB HRMS m/z calcd. for $C_{25}H_{37}O_3SiS$ $(M+H)^+$ 445.2233, found 445.2215.

4-Hydroxy-2-[2,2-(2,6-dimethyl-1,5-heptadienyl)-*p*-toluenesulfonylethyl]butenolide. To a solution of the furan derivative obtained above (1.12 g, 2.51 mmol) in dichloromethane (20 mL) was added a catalytic amount of tetraphenylporphine (TPP), and the solution was cooled at -78 °C. The mixture was irradiated with a halogen lamp (Riko-HGA-300A) at -78 °C under an oxygen atmosphere, and the reaction was monitored by TLC. After the starting material was consumed, the reaction mixture was flushed with argon. The solvent was removed *in vacuo* and the crude products were purified by column chromatography on silica gel (from 2 % to 50 % ethyl acetate in hexane) to give the corresponding γ-hydroxybutenolide (802 mg, 82 %) as a mixture of stereoisomers: IR (nujol, cm^{-1}); 3392, 1762, 1596, 1442, 1286, 1140; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22 (brs, 3H), 1.58 (s, 3H), 1.68 (s, 3H), 1.93 (brs, 4H), 2.44 (s, 3H), 2.65 (dd, 1H, J = 15.2, 8.9 Hz), 3.13 (dd, 1H, J = 15.2, 5.2 Hz), 4.31 (brs, 1H), 4.57 (brs, 1H), 4.90 (brs, 1/2×1H), 4.92 (brs, 1/2×1H), 4.99 (brs, 1H), 6.06 (brs, 1H), 6.92 (brs, 1H), 7.32 (d, 2H, J = 8.3 Hz), 7.71 (d, 2H, J = 8.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.42, 16.54, 17.62, 21.60, 24.49, 24.70, 25.60, 26.08, 39.53, 61.53, 61.91, 96.96, 97.00, 115.77, 115.81, 123.32, 123.37, 129.16, 129.22, 129.51, 132.06, 132.09, 133.10, 133.33, 134.00, 134.09, 144.88, 145.91, 146.43, 146.84, 146.97, 171.09; FAB HRMS m/z calcd. for $C_{22}H_{29}O_5S$ $(M+H)^+$ 405.1735, found 405.1735.

Methyl (E,E,E)-4-oxo-2-[(2,6-dimethyl-1,5-heptadienyl)vinyl]but-2-enoate (D) and methyl (Z,E,E)-4-oxo-2-[(2,6-dimethyl-1,5-heptadienyl)vinyl]but-2-enoate. To a solution of the γ-hydroxybutenolide obtained above (598 mg, 1.53 mmol) in DMSO (10 mL) was added N,N'-diisopropylethylamine (0.80 mL, 4.58 mmol) at room temperature. The reaction mixture was stirred for 3 h and methyl iodide (1.14 mL, 18.3 mmol) was added at the same temperature. After the mixture was stirred for 1 h, saturated aqueous NH_4Cl solution was added, and the resulting mixture was extracted with ether. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* to give the corresponding aldehyde.

To a THF (10 mL) solution of the crude aldehyde was added diazabicycloundecene (0.21 mL, 1.44 mmol) at 0 °C. After the reaction mixture was stirred at 0 °C for 30 min, saturated aqueous NH_4Cl solution was added, and the resulting mixture was extracted with ether. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* to give the crude products. Column chromatography on silica gel (5 % ethyl acetare in hexane) gave 1 : 6 mixture of **D** and its (Z)-stereoisomer (219 mg, 54 % for 2 steps). These compounds were separated by column chromatography on silica gel (1 % ethyl acetate in hexane): Data for (Z)-stereoisomer: IR (neat, cm^{-1}); 1734, 1674, 1630, 1588, 1440, 1214, 1158; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.61 (s, 3H), 1.69 (s, 3H), 1.85 (s, 3H), 2.16 (brs, 4H), 3.95 (s, 3H), 5.07 (brs, 1H), 6.01 (d, 1H, J = 11.2 Hz), 6.08 (d, 1H, J = 7.8 Hz), 6.23 (d, 1H, J = 15.4 Hz), 6.92 (dd, 1H, J = 11.2, 15.4 Hz), 9.75 (d, 1H, J = 7.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.50, 17.70, 25.67, 26.34, 40.38, 52.60, 123.25, 124.85, 125.56, 127.68, 132.30, 136.42, 148.82, 150.34, 166.68, 190.58. Data for (E)-stereoisomer **D**: IR (neat, cm^{-1}); 1732, 1672, 1632, 1588, 1440, 1244, 1142; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.62 (s, 3H), 1.69 (s, 3H), 1.86 (d, 3H,

J = 1.2 Hz), 2.17 (brs, 4H), 3.87 (s, 3H), 5.06-5.10 (brm, 1H), 6.07 (d, 1H, J = 11.0 Hz), 6.50 (d, 1H, J = 7.3 Hz), 6.79 (d, 1H, J = 15.1 Hz), 7.07 (dd, 1H, J = 11.0, 15.1 Hz), 10.11 (d, 1H, J = 7.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.46, 17.68, 25.66, 26.35, 40.30, 52.67, 120.13, 123.33, 125.05, 129.65, 132.23, 138.74, 146.21, 148.24, 167.10, 191.16; FAB HRMS m/z calcd. for $C_{16}H_{23}O_3$ $(M+H)^+$ 263.1647, found 263.1632.

(E)-1-(1-Buten-3-ynyl)-2,5,5,8a-tetramethyl-*trans*-3,4,4a,5,6,7,8,8a-octahydronaphthalene. To a THF (25 mL) and HMPA (2.5 mL) solution of 1-*p*-toluenesulfonylmethyl-2,5,5,8a-tetramethyloctahydronaphthalene (1.60 g, 4.43 mmol) prepared from the corresponding alcohol[27] was added dropwise *n*-butyllithium (1.6 M solution in hexane, 3.33 mL, 5.32 mmol) at -78 °C. The reaction mixture was stirred at -78 °C for 20 min and propargyl bromide (0.790 mL, 8.86 mmol) was added at the same temperature. After the mixture was gradually warmed to room temperature and stirred for an additional 40 min, saturated aqueous NH_4Cl solution was added, and the resulting mixture was extracted with ether. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* to give the corresponding coupling products.

To a THF (35 mL) solution of the crude products was added potassium *tert*-butoxide (1.59 g, 14.2 mmol) at 0 °C. After the reaction mixture was stirred at room temperature for 30 min, saturated aqueous NH_4Cl solution was added, and the resulting mixture was extracted with ether. The organic layers were combined, washed with saturated aqueous NH_4Cl solution, brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* to give the crude products. Column chromatography on silica gel (hexane) gave the corresponding acetylene (849 mg, 79 % for 2 steps): IR (neat, cm^{-1}); 3312, 2100, 1460, 1378, 960; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.838 (s, 3H), 0.887 (s, 3H), 0.997 (s, 3H), 1.06-1.19 (m, 3H), 1.38-1.49 (m, 3H), 1.52-1.61 (m, 1H), 1.63-1.70 (m, 2H), 1.66 (s, 3H), 2.07-2.10 (m, 2H), 2.91 (d, 1H, J = 2.4 Hz), 5.36 (dd, 1H, J = 16.4, 2.4 Hz), 6.59 (d, 1H, J = 16.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.69, 18.93, 20.23, 21.16, 21.53, 33.18, 33.29, 33.73, 38.00, 38.14, 41.64, 51.10, 76.78, 83.16, 111.43, 129.62, 140.41, 142.88; FAB HRMS m/z calcd. for $C_{18}H_{26}$ (M^+) 242.2035, found 242.2074.

(E)-5-(2,5,5,8a-Tetramethyl-*trans*-3,4,4a,5,6,7,8,8a-octahydronaphthyl)pent-4-en-2-yn-1-ol. To a THF (20 mL) solution of the acetylene obtained above (658 mg, 2.71 mmol) was added dropwise *n*-butyllithium (1.6 M solution in hexane, 2.04 mL, 3.26 mmol) at -78 °C over 20 min. The reaction mixture was stirred at -78 °C for an additional 20 min and paraformaldehyde (411 mg, 13.6 mmol) was added at this temperature. After the mixture was warmed to room temperature and stirred for 2 h, saturated aqueous NH_4Cl solution was added, and the resulting mixture was extracted with ether. The organic layers were combined, washed with saturated aqueous NH_4Cl solution, brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* to give the crude products. Column chromatography on silica gel (2 % ether in hexane) gave the corresponding alcohol (614 mg, 83 %): IR (disk, cm^{-1}) 3344, 2208, 1446, 1376, 1010; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.84 (s, 3H), 0.88 (s, 3H), 0.99 (s, 3H), 1.06-1.21 (m, 3H), 1.38-1.49 (m, 3H), 1.52-1.69 (m, 3H), 1.65 (s, 3H), 2.06-2.09 (m, 2H), 4.41 (d, 2H, J = 1.6 Hz), 5.39 (dd, 1H, J = 16.4, 1.6 Hz), 6.51 (d, 1H, J = 16.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.69, 18.92, 20.23, 21.17, 21.52, 33.17, 33.28, 33.71, 38.00, 38.15, 41.62, 51.09, 51.70, 85.16, 86.90, 111.81, 129.46, 140.51, 141.72; FAB HRMS m/z calcd. for $C_{19}H_{28}O$ (M^+) 272.2141, found 272.2140.

(E,E)-4-Hydroxy-2-[(2,5,5,8a-tetramethyl-*trans*-3,4,4a,5,6,7,8,8a-octahydronaphthyl) vinyl]but-2-enoic acid. To a 1.0 M solution of *iso*butylmagnesium chloride in ether (1.84 mL, 1.84 mmol) prepared from *iso*butyl chloride (22.4 mL, 214 mmol), magnesium turnings (5.45 g, 224 mmol), and ether (150 mL) was added titanocene dichloride (14 mg, 0.0551 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 20 min and a ether (1 mL) solution of the alcohol obtained above (100 mg, 0.367 mmol) was added dropwise at the same temperature over 10 min. The mixture was warmed to room temperature and stirred for an additional 2.5 h. The bulk of ether was removed *in vacuo*, and THF (3 mL) was added. After stirring at 0 °C for 30 min under an atmosphere of carbon dioxide, 1 N HCl solution was added, and the resulting mixture was extracted with ethyl acetate. The organic layers were combined, washed with 1 N HCl solution, brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* to give the crude products. Column chromatography on silica gel (from 17 % to 50 % ethyl acetate in hexane) gave the corresponding acid (85 mg, 73 %): IR (disk, cm^{-1}) 3388 (br), 2932, 1694, 1446, 1376, 1262, 974; ^1H NMR (400 MHz, $CDCl_3$) δ 0.846 (s, 3H), 0.894 (s, 3H), 1.01 (s, 3H), 1.11-1.19 (m, 2H), 1.38-1.70 (m, 7H), 1.69 (s, 3H), 2.08-2.11 (m, 2H), 4.52 (d, 2H, J = 6.1 Hz), 6.01 (d, 1H, J = 16.3 Hz), 6.15 (d, 1H, J = 16.3 Hz), 6.92 (t, 1H, J = 6.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.79, 19.00, 20.30, 21.40, 21.54, 33.19, 33.31, 33.67, 37.99, 38.24, 41.67, 51.17, 60.33, 125.25, 128.57, 130.44, 134.94, 141.19, 141.73, 171.93; FAB HRMS m/z calcd. for $C_{20}H_{31}O_3$ $(M+H)^+$ 319.2273, found 319.2255.

Methyl (E,E)-4-oxo-2-[(2,5,5,8a-tetramethyl-*trans*-3,4,4a,5,6,7,8,8a-octahydronaphthyl) vinyl]but-2-enoate (E). To a solution of the acid obtained above (94 mg, 0.295 mmol) in benzene (3 mL) was added N,N,N',N'-tetramethylguanidine (0.074 mL, 0.590 mmol) at room temperature. The reaction mixture was stirred for 1.5 h and methyl iodide (0.184 mL, 2.95 mmol) was added at the same temperature. After stirring at room temperature for 2 h, the reaction mixture was filtered and concentrated *in vacuo* to give the corresponding methyl ester.

To a solution of the crude methyl ester in dichloromethane (3 mL) was added manganese dioxide (2.82 g) at room temperature, and the mixture was stirred for 15 min. The reaction mixture was filtered and concentrated *in vacuo* to give the crude products which were purified by column chromatography on silica gel (2 % ether in hexane) to afford **E** (58 mg, 60 % for 2 steps): IR (neat, cm^{-1}) 2928, 1732, 1678, 1608, 1438, 1246; ^1H NMR (400 MHz, $CDCl_3$) δ 0.854 (s, 3H), 0.902 (s, 3H), 1.05 (s, 3H), 1.11-1.20 (m, 3H), 1.39-1.51 (m, 3H), 1.53-1.72 (m, 3H), 1.74 (s, 3H), 2.13-2.15 (m, 2H), 3.86 (s, 3H), 6.54 (s, 2H), 6.62 (d, 1H, J = 7.6 Hz), 10.05 (d, 1H, J = 7.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.66, 18.96, 20.55, 21.53, 21.55, 33.18, 33.33, 33.95, 38.17, 38.35, 41.57, 51.07, 52.75, 123.99, 131.25, 131.79, 141.02, 141.89, 146.54, 167.15, 191.51; FAB HRMS m/z calcd. for $C_{21}H_{31}O_3$ $(M+H)^+$ 331.2273, found 331.2272.

3-[2,2-(2,6,6-Trimethylcyclohex-1-enyl)-*p*-toluenesulfonylethyl]-5-trimethylsilylfuran. To a THF (18 mL) solution of cyclocitryl sulfone **4** (661 mg, 2.26 mmol) was added dropwise *n*-butyllithium (1.6 M solution in hexane, 1.69 mL, 2.71 mmol) at -78 °C. The reaction mixture was stirred at -78 °C for 20 min and a THF (2.0 mL) solution of chloride derived from 3-chloromethyl-5-trimethylsilylfuran [17,28] (558 mg, 2.94 mmol) was added at the same temperature. After the mixture was gradually warmed to room temperature and stirred for an additional 12 h, H_2O was added, and the resulting mixture was extracted with ether. The organic layers were combined, washed with H_2O, brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* to give the crude

products. Column chromatography on silica gel (1 % ethyl acetate in hexane) gave the corresponding furan derivative (651 mg, 65 %): IR (nujol, cm^{-1}); 1598, 1470, 1386, 1298, 1292, 1250, 1138, 1084, 848; ^1H NMR (400 MHz, $CDCl_3$) δ 0.17 (s, 9H), 0.64 (s, 3H), 1.04 (s, 3H), 1.38-1.44 (m, 2H), 1.48-1.63 (m, 2H), 2.00-2.21 (m, 2H), 2.05 (s, 3H), 2.41 (s, 3H), 2.99 (dd, 1H, J = 15.1, 6.0 Hz), 3.43 (dd, 1H, J = 15.1, 7.6 Hz), 3.91 (dd, 1H, J = 7.6, 6.0 Hz), 6.05 (s, 1H), 7.23 (d, 2H, J = 8.1 Hz), 7.30 (s, 1H), 7.61 (d, 2H, J = 8.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ -1.75, 19.00, 21.55, 23.27, 26.59, 27.78, 29.04, 34.49, 36.00, 39.58, 68.79, 120.74, 121.25, 128.17, 129.42, 130.88, 137.59, 139.49, 143.55, 144.54, 160.55; FAB HRMS m/z calcd. for $C_{25}H_{37}O_3SiS$ $(M+H)^+$ 445.2232, found 445.2237.

4-Hydroxy-3-[2,2-(2,6,6-trimethylcyclohex-1-enyl)-*p*-toluenesulfonylethyl]butenolide. To a solution of the furan derivative obtained above (400 mg, 0.90 mmol) in dichloromethane (9.0 mL) was added a catalytic amount of tetraphenylporphine (TPP), and the solution was cooled at -78 °C. The mixture was irradiated with a halogen lamp (Riko-HGA-300A) at -78 °C under an oxygen atmosphere, and the reaction was monitored by TLC. After the starting material was consumed, the reaction mixture was flushed with argon. The solvent was removed *in vacuo* and the crude products were purified by column chromatography on silica gel (from 2 % to 50 % ethyl acetate in hexane) to give the corresponding γ-hydroxybutenolide (338 mg, 96 %) as a mixture of stereoisomers: IR (nujol, cm^{-1}); 3428, 1774, 1659, 1600, 1460, 1298, 1142; ^1H NMR (400 MHz, $CDCl_3$) δ 0.67 (s, 1/2×3H), 0.85 (s, 1/2×3H), 0.86 (s, 1/2×3H), 0.87 (s, 1/2×3H), 1.34-1.55 (m, 4H), 1.99-2.11 (m, 2H), 1.99 (s, 1/2×3H), 2.01 (s, 1/2×3H), 2.44 (s, 3H), 2.84 (brd, 1/2×1H, J = 17.1 Hz), 2.92 (dd, 1/2×1H, J = 16.6, 2.7 Hz), 3.64 (dd, 1/2×1H, J = 16.6, 8.8 Hz), 3.74 (dd, 1/2×1H, J = 16.8, 9.8 Hz), 4.09 (dd, 1/2×1H, J = 8.8, 4.8 Hz), 4.28 (dd, 1/2×1H, J = 9.8, 3.2 Hz), 5.07 (brs, 1/2×1H), 5.08 (brs, 1/2×1H), 5.75 (brs, 1H), 5.79 (brs, 1/2×1H), 6.01 (brd, 1/2×1H, J = 7.6 Hz), 7.32 (d, 1/2×2H, J = 6.4 Hz), 7.33 (d, 1/2×2H, J = 6.4 Hz), 7.66 (d, 1/2×2H, J = 8.3 Hz), 7.69 (d, 1/2×2H, J = 8.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.75, 18.80, 21.61 23.20, 23.29, 27.93, 28.43, 28.53, 28.64, 29.79, 30.26, 34.52, 34.57, 36.18, 36.37, 39.38, 39.41, 65.46, 65.81, 98.65, 99.02, 120.14, 120.37, 128.65, 128.91, 129.61, 129.81, 130.78, 131.27, 136.63, 137.46, 138.65, 139.04, 145.16, 145.30, 164.08, 164.82, 170.14, 170.27; FAB HRMS m/z calcd. for $C_{22}H_{29}O_5S$ $(M+H)^+$ 405.1735, found 405.1748.

(E,E)-3-Methoxycarbonyl-2-[(2,6,6-trimethylcyclohex-1-enyl)vinyl]-2-propenal (G). To a solution of the γ-hydroxybutenolide obtained above (100 mg, 0.255 mmol) in DMF (2.5 mL) was added diazabicycloundecene (0.15 mL, 1.02 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 7 h and methyl iodide (0.19 mL, 3.07 mmol) was added at the same temperature. After the mixture was stirred at room temperature for 1 h, saturated aqueous NH_4Cl solution was added, and the resulting mixture was extracted with ether. The organic layers were combined, washed with saturated $NaHCO_3$ solution, brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give the crude products. Column chromatography on silica gel (2 % ethyl acetate in hexane) gave **G** (38 mg, 57 %): IR (neat, cm^{-1}); 1712, 1600, 1440, 1218, 1172; ^1H NMR (400 MHz, $CDCl_3$) δ 1.09 (s, 6H), 1.46-1.49 (m, 2H), 1.59-1.65 (m, 2H), 1.80 (s, 3H), 2.06 (t, 2H, J = 6.2 Hz), 3.81 (s, 3H), 6.21 (s, 1H), 7.17 (d, 1H, J = 16.8 Hz), 7.39 (d, 1H, J = 16.8 Hz), 9.74 (d, 1H, J = 2.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.04, 21.76, 28.86, 33.45, 34.12, 39.81, 51.92, 122.96, 128.95, 133.62, 137.88, 140.14, 146.26, 166.12, 193.97; FAB HRMS m/z calcd. for $C_{16}H_{22}O_3$ (M^+) 262.1569, found 262.1573.

3-[2-(2,6,6-Trimethylcyclohex-1-enyl)ethyl]-2-trimethylsilylfuran.[17] To a methanol (60 mL) solution of **6** (4.0 g, 9.0 mmol) was added 5 % Na (Hg) (18.0 g, 42.8 mmol) and dibasic sodium phosphate (5.11 g, 36.0 mmol), and then the mixture was stirred under the reflux condition for 2 h. The reaction mixture was filtered and the filtrate was poured into 3 % NaOH solution, and extracted with hexane. The organic layers were combined, washed with water, brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* to give the crude products. Column chromatography on silica gel (hexane) followed by distillation (1 mmHg, 90-100 °C) gave the title compound (2.43 g, 93 % yield): IR (neat, cm^{-1}); 1572, 1250, 1096, 1062, 842; ^1H NMR (400 MHz, $CDCl_3$) δ 0.29 (s, 9H), 1.03 (s, 6H), 1.43-1.46 (m, 2H), 1.56-1.62 (m, 2H), 1.66 (s, 3H), 1.94 (t, 2H, J = 6.3 Hz), 2.20-2.24 (m, 2H), 2.51-2.55 (m, 2H), 6.36 (d, 1H, J = 1.0 Hz), 7.55 (d, 1H, J = 1.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 0.00, 20.40, 20.91, 27.11, 29.56, 31.18, 33.66, 35.85, 40.68, 111.77, 128.43, 136.69, 137.81, 146.88, 154.72.

4-Hydroxy-2-[2-(2,6,6-trimethylcyclohex-1-enyl)ethyl]butenolide.[17] To a solution of the desulfonated furan derivative (500 mg, 1.72 mmol) in dichloromethane (10 mL) was added a catalytic amount of tetraphenylporphine (TPP), and the solution was cooled at -78 °C. The mixture was irradiated with a halogen lamp (Riko-HGA-300A) at -78 °C under an oxygen atmosphere, and the reaction was monitored by TLC. After the starting material was consumed, the reaction mixture was flushed with argon. The solvent was removed *in vacuo* and the crude products were purified by column chromatography on silica gel (from 2 % to 50 % ethyl acetate in hexane) to give the corresponding γ-hydroxybutenolide (275 mg, 64 %): IR (nujol, cm^{-1}); 3316, 1734, 1462; ^1H NMR (400 MHz, $CDCl_3$) δ 1.00 (s, 6H), 1.41-1.44 (m, 2H), 1.54-1.60 (m, 2H), 1.62 (s, 3H), 1.92 (t, 2H, J = 6.2 Hz), 2.19-2.23 (m, 2H), 2.32-2.36 (m, 2H), 4.47 (brs, 1H), 6.13 (brs, 1H), 6.89 (d, 1H, J = 1.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.39, 19.81, 25.88, 26.31, 28.49, 32.70, 34.93, 39.68, 96.93, 128.58, 135.62, 138.57, 143.03, 172.05.

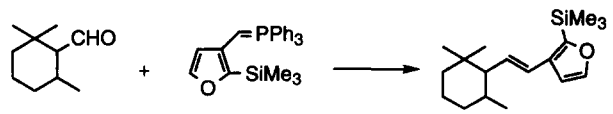
Methyl (E)-4-oxo-2-[(2,6,6-trimethylcyclohex-1-enyl)ethyl]but-2-enoate (H) and methyl (Z)-4-oxo-2-[(2,6,6-trimethylcyclohex-1-enyl)ethyl]but-2-enoate. To a solution of the γ-hydroxybutenolide obtained above (50 mg, 0.20 mmol) in DMSO (2.0 mL) was added N,N'-diisopropylethylamine (0.10 mL, 0.599 mmol) at room temperature. The reaction mixture was stirred at room temperature for 3 h and methyl iodide (0.15 mL, 2.40 mmol) was added at the same temperature. After the mixture was stirred at room temperature for 1 h, saturated aqueous NH_4Cl solution was added, and the resulting mixture was extracted with ether. The organic layers were combined, washed with saturated $NaHCO_3$ solution, brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* to give the crude products. Column chromatography on silica gel (5 % ethyl acetate in hexane) gave 1 : 3 mixture of the compound **H** and its (Z)-stereoisomer (30 mg, 57 %). These compounds were separated by column chromatography on silica gel (1 % ethyl acetate in hexane): Data for (Z)-stereoisomer: IR (neat, cm^{-1}); 1730, 1684, 1626, 1438, 1220; ^1H NMR (400 MHz, $CDCl_3$) δ 1.00 (s, 6H), 1.42-1.45 (m, 2H), 1.55-1.60 (m, 2H), 1.61 (s, 3H), 1.92 (t, 2H, J = 6.2 Hz), 2.15-2.19 (m, 2H), 2.48-2.53 (m, 2H), 3.89 (s, 3H), 6.16 (dt, 1H, J = 7.6, 1.2 Hz), 10.06 (d, 1H, J = 7.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.34, 19.69, 27.18, 28.42, 32.70, 34.94, 35.17, 39.62, 52.43, 128.77, 133.61, 135.33, 151.85, 166.74, 192.07. Data for (E)-stereoisomer **H**: IR (neat, cm^{-1}); 1724, 1682, 1440, 1260; ^1H NMR (400 MHz, $CDCl_3$) δ 1.03 (s, 6H), 1.43-1.46 (m, 2H), 1.57-1.61 (m, 2H), 1.69 (s, 3H), 1.94 (t, 2H, J = 6.3 Hz), 2.18-2.22 (m, 2H), 2.80-2.85 (m, 2H), 3.86 (s, 3H), 6.77 (d, 1H, J = 7.8 Hz), 10.19 (d, 1H, J = 7.8 Hz);

^{13}C NMR (100 MHz, $CDCl_3$) δ 19.39, 20.05, 27.90, 28.57, 29.43, 32.80, 34.98, 39.71, 52.69, 129.24, 133.40, 133.69, 150.14, 166.82, 191.61; FAB HRMS m/z calcd. for $C_{16}H_{24}O_3$ (M^+) 264.1725, found 264.1743.

3-(2-Trimethylsilylfuryl)methyltriphenylphosphonium bromide. To a ether (30 mL) solution of 2-silyl-3-furanmethanol[17, 28] (500 mg, 2.94 mmol) and pyridine (0.0356 mL, 0.441 mmol) was added phosphorous tribromide (0.206 mL, 3.53 mmol) at 0 °C. After the reaction mixture was stirred at room temperature for 80 min, H_2O was added, and the resulting mixture was extracted with ether. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* to give the corresponding bromide.

To a benzene (30 mL) solution of the crude bromide was added triphenylphosphine (1.16 g, 4.41 mmol) at room temperature. After stirring for 4 h under the reflux condition, the reaction mixture was filtered to give the corresponding triphenylphosphonium salt (691 mg, 47 %): FAB HRMS m/z calcd. for $C_{26}H_{28}OSiP$ $(M\text{-}Br)^+$ 415.1647, found 415.1638.

(E)-3-[2-(1,6-*trans*)-(2,2,6-Trimethylcyclohexyl)vinyl]-2-trimethylsilylfuran. To a solution of the phosphonium salt obtained(1.64 g, 2.90 mmol) in ether (20 mL) was added dropwise *n*-butyllithium (1.6 M solution in hexane, 1.81 mL, 2.90 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 10 min and a ether (5 mL) solution of *cis*-dihydrocyclocitral[29] (300 mg, 1.93 mmol) was added at this temperature. After the mixture was gradually warmed to room temperature and stirred for an additional 30 min, H_2O was added, and the resulting mixture was extracted with ethyl acetate. The organic layers were combined, washed with saturated aqueous NH_4Cl solution, brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* to give the crude products. Column chromatography on silica gel (hexane) to give the corresponding furan derivative (267 mg, 48 %): IR (neat, cm^{-1}) 2960, 1458, 1250, 968, 840, 634; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.304 (s, 9H), 0.801 (d, 3H, J = 6.3 Hz), 0.861 (s, 6H), 0.90-0.98 (m, 1H), 1.19-1.23 (m, 1H), 1.40-1.53 (m, 5H), 1.72-1.76 (m, 1H), 5.64 (dd, 1H, J = 15.6, 9.4 Hz), 6.29 (d, 1H, J = 15.6 Hz), 6.52 (d, 1H, J = 2.0 Hz), 7.50 (dd, 1H, J = 1.7, 0.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 0.00, 21.50, 22.69, 23.05, 32.46, 32.92, 34.98, 36.96, 42.56, 60.06, 108.68, 123.36, 133.06, 135.21, 147.20, 156.68; FAB HRMS m/z calcd. for $C_{18}H_{30}OSi$ (M^+) 290.2066, found 290.2062.



Methyl (Z,E)-4-oxo-2-[(1,6-*trans*)-(2,2,6-trimethylcyclohexyl)vinyl]but-2-enoate. To a solution of the furan derivative (200 mg, 0.688 mmol) obtained above in dichloromethane (6 mL) was added a catalytic amount of tetraphenylporphine (TPP), and the solution was cooled to -78 °C. The mixture was irradiated with a halogen lamp (Riko-HGA-300A) at -78 °C under an oxygen atmosphere, and the reaction was monitored by TLC. After the starting material was consumed, the reaction mixture was flushed with argon. The solvent was removed *in vacuo* and the residue was roughly purified by column chromatography on silica gel (from 2 % to 50 % ethyl acetate in hexane) to give the corresponding γ-hydroxybutenolide.

To a solution of the γ-hydroxybutenolide obtained in DMSO (6 mL) was added N,N'-diisopropylethylamine (0.360 mL, 2.07 mmol) at room temperature. The reaction mixture was stirred at room temperature for 1.5 h and methyl iodide (0.514 mL, 8.26 mmol) was added at the same temperature. After the mixture was stirred at room temperature for 5 min, saturated aqueous NH_4Cl solution was added, and the resulting mixture was extracted with ethyl acetate. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* to give the crude products. Column chromatography on silica gel (17 % ether in hexane) gave the corresponding methoxycarbonyldienal compound (150 mg, 83 % for 2 steps): IR (neat, cm^{-1}) 2928, 1738, 1680, 1624, 1214, 1162; ^1H NMR (400 MHz, $CDCl_3$) δ 0.761 (d, 3H, J = 6.1 Hz), 0.839 (s, 3H), 0.847 (s, 3H), 1.14-1.22 (m, 1H), 1.40-1.55(m, 6H), 1.71-1.75 (m, 1H), 3.92(s, 3H), 5.99 (dd, 1H, J = 9.5, 15.6 Hz), 6.03 (d, 1H, J = 7.6 Hz), 6.14 (d, 1H, J = 15.9 Hz), 9.76 (d, 1H, J = 7.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.52, 21.57, 21.80, 31.42, 31,70, 34.31, 35.20, 41.22, 52.54, 59.53, 127.85, 128.02, 146.21, 149.57, 166.63, 190.91; FAB HRMS m/z calcd. for $C_{16}H_{25}O_3$ $(M+H)^+$ 265.1804, found 265.1809.

Methyl (E,E)-4-oxo-2-[(1,6-*trans*)-(2,2,6-trimethylcyclohexyl)vinyl]but-2-enoate (I). To a solution of the methoxycarbonyldienal compound (130 mg, 0.492 mmol) in benzene (4 mL) was added a catalytic amount of iodine. After the reaction mixture was irradiated by fluorescence lamp at room temperature for 1h, saturated aqueous $Na_2S_2O_3$ solution was added, and the resulting mixture was extracted with ether. The organic layers were combined, washed with aqueous $Na_2S_2O_3$ solution, brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* to give the crude products. Column chromatography on silica gel (10 % ether in hexane) gave 1 : 2 mixture of **I** and its (Z)-stereoisomer (121 mg, 93 %). These stereoisomers were separated by column chromatography on silica gel (1 % ether in hexane): IR (neat, cm^{-1}) 2924, 1732, 1680, 1440, 1246, 1128, 976; ^1H NMR (400 MHz, $CDCl_3$) δ 0.809 (d, 3H, J = 6.1 Hz), 0.868 (s, 3H), 0.886 (s, 3H), 1.16-1.26 (m, 1H), 1.42-1.60 (m, 6H), 1.73-1.77 (m, 1H), 3.85 (s, 3H), 5.97 (dd, 1H, J = 15.6, 9.6 Hz), 6.54 (d, 1H, J = 15.6 Hz), 6.59 (d, 1H, J = 7.6 Hz), 10.10 (d, 1H, J = 7.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.61, 21.68, 21.80, 31.49, 31.57, 34.13, 35.29, 41.27, 52.71, 59.97, 122.71, 131.22, 146.18, 147.92, 167.11, 191.64; FAB HRMS m/z calcd. for $C_{16}H_{25}O_3$ $(M+H)^+$ 265.1804, found 265.1815.

Representative procedure of the 1,2-dihydropyridine derivatives (DHPx). To a dioxane-d_8 (0.5 mL) solution of compound **A** (11 mg, 0.0435 mmol) and tetramethylsilane as a standard substrate was added *n*-propylamine (4.3 μL, 0.0522 mmol) at room temperature. The reaction was monitored by NMR. 1,2-Dihydropyridine derivative was quantitatively produced within 5 min. After the completion of the reaction, the mixture was concentrated *in vacuo* at 0 °C, and IR and Mass spectra were measured.

DHP_A: IR (neat, cm^{-1}) 1724, 1640, 1580, 1466, 1442, 1262; ^1H NMR (400 MHz, $C_4D_8O_2$) δ 0.84 (t, 3H, J = 7.3 Hz), 1.00 (s, 3H), 1.04 (s, 3H), 1.42-1.62 (m, 6H), 1.76 (s, 3H), 2.01-2.07 (m, 2H), 2.52-2.60 (m, 1H), 2.75-2.81 (m, 1H), 3.62 (s, 3H), 4.68 (dd, 1H, J = 1.6 Hz, 7.6 Hz), 5.27 (d, 1H, J = 4.0 Hz), 5.63 (ddd, 1H, J = 0.4, 1.7, 4.0 Hz), 5.89 (dd, 1H, J = 0.4, 7.6 Hz); ^{13}C NMR (100 MHz, $C_4D_8O_2$) δ 11.35, 19.98, 20.74, 21.14, 27.33, 28.92, 35.25, 35.53, 41.03, 51.49, 53.75, 57.20, 86.83, 123.79, 126.78, 137.60, 137.98, 142.97, 166.14; FAB HRMS m/z calcd. for $C_{19}H_{30}O_2N$ $(M+H)^+$ 304.2277, found 304.2284.

DHP_C: ^1H NMR (400 MHz, $C_4D_8O_2$) δ 0.876 (t, 3H, J = 7.2 Hz), 1.51 (q, 2H, J = 7.2 Hz), 1.66 (s, 3H), 1.68 (s, 3H), 2.75-2.82 (m, 1H), 2.94-3.01 (m, 1H), 3.62 (s, 3H), 4.78 (dd, 1H, J = 9.2, 6.0 Hz), 4.88 (dd,

1H, J = 7.6, 2.0 Hz), 5.59 (dm, 1H, J = 9.2 Hz), 5.74 (ddd, 1H, J = 6.0, 2.0, 0.8 Hz), 6.04 (d, 1H, J = 7.6 Hz); ^{13}C NMR (100 MHz, $C_4D_8O_2$) δ 11.37, 17.68, 22.25, 25.60, 51.44, 54.54, 55.03, 90.90, 120.34, 123.57, 130.14, 136.46, 138.14, 166.26; FAB HRMS m/z calcd. for $C_{14}H_{22}O_2N$ $(M+H)^+$ 236.1651, found 236.1665.

DHP$_D$: ^{1}H NMR (400 MHz, $C_4D_8O_2$) δ 0.876 (t, 3H, J = 7.3 Hz), 1.51 (q, 2H, J = 7.3 Hz), 1.58 (s, 3H), 1.64 (d, 3H, J = 1.0 Hz), 1.66 (d, 3H, J = 1.2 Hz), 1.97-2.03 (m, 2H), 2.06-2.14 (m, 2H), 2.74-2.81 (m, 1H), 2.94-3.01 (m, 1H), 3.63 (s, 3H), 4.81 (dd, 1H, J = 9.3, 5.9 Hz), 4.87 (dd, 1H, J = 7.3, 2.0 Hz), 5.05 (t, 1H, J = 7.1 Hz), 5.58 (dd, 1H, J = 9.3, 1.2 Hz), 5.73 (dd, 1H, J = 5.9, 2.0 Hz), 6.03 (d, 1H, J = 7.3 Hz); ^{13}C NMR (100 MHz, $C_4D_8O_2$) δ 11.44, 16.04, 17.69, 22.21, 25.74, 26.76, 39.94, 51.44, 54.57, 55.09, 90.84, 120.50, 123.60, 124.76, 128.11, 131.85, 135.97, 138.20, 166.25; FAB HRMS m/z calcd. for $C_{19}H_{30}O_2N$ $(M+H)^+$ 304.2277, found 304.2240.

DHP$_E$: IR (neat, cm^{-1}) 1724, 1580, 1466, 1260, 1098, 1080; ^{1}H NMR (400 MHz, $C_4D_8O_2$) δ 0.812-0.958 (m, 9H), 1.00 (s, 1/4×3H), 1.02 (s, 3/4×3H), 1.11-1.67 (m, 11H), 1.70 (s, 3H), 2.09-2.11 (m, 2H), 2.50-2.64 (m, 1H), 2.75-2.82 (m, 1H), 3.61 (s, 3H), 4.66 (dd, 3/4×1H, J = 7.6, 1.6 Hz), 4.69 (dd, 1/4×1H, J = 7.6, 1.6 Hz), 5.23 (brd, 3/4×1H, J = 3.2 Hz), 5.37 (m, 1/4×1H), 5.55 (brd, 1/4×1H, J = 3.2 Hz), 5.65 (brd, 3/4 ×1H, J = 3.2 Hz), 5.87 (d, 1H, J = 7.6 Hz); ^{13}C NMR (100 MHz, $C_4D_8O_2$) δ 11.26, 11.35, 18.95, 19.47, 19.64, 20.62, 20.76, 21.72, 22.15, 33.83, 36.18, 36.69, 37.37, 39.72, 42.00, 42.34, 51.47, 52.57, 52.74, 53.46, 54.08, 56.21, 86.57, 87.55, 123.42, 125.85, 126.62, 133.57, 137.85, 138.29, 147.55, 166.16; FAB HRMS m/z calcd. for $C_{24}H_{38}O_2N$ $(M+H)^+$ 372.2902, found 372.2893.

Representative procedure of the Schiff base derivatives (SCFx). To a dioxane-d_8 (0.5 mL) solution of **B** (17 mg, 0.0648 mmol) and tetramethylsilane as a standard substrate was added *n*-propylamine (6.4 μL, 0.0778 mmol) at room temperature. The reaction was monitored by NMR. The corresponding Schiff base derivative was quantitatively produced within 10 min. After the completion of the reaction, the mixture was concentrated *in vacuo* at 0 °C, and IR and Mass spectra were measured.

SCF$_B$: IR (neat, cm^{-1}) 1726, 1634, 1580, 1440, 1382, 1362; ^{1}H NMR (400 MHz, $C_4D_8O_2$) δ 0.890 (t, 3H, J = 7.6 Hz), 1.02 (s, 6H), 1.45-1.48 (m, 2H), 1.55-1.64 (m, 4H), 1.71 (d, 3H, J = 0.7 Hz), 2.03 (t, 2H, J = 6.1 Hz), 3.42 (td, 2H, J = 6.8, 1.2 Hz), 3.81 (s, 3H), 6.16 (d, 1H, J = 16.1 Hz), 6.38-6.42 (d, 1H), 6.41 (d, 1H, J = 9.3 Hz), 8.11 (dt, 1H, J = 9.3, 1.2 Hz); ^{13}C NMR (100 MHz, $C_4D_8O_2$) δ 12.02, 19.68, 21.68, 24.63, 29.08, 33.52, 34.78, 40.14, 51.98, 64.16, 130.87, 131.74, 132.20, 133.60, 137.98, 140.86, 159.37, 167.57; FAB HRMS m/z calcd. for $C_{19}H_{30}O_2N$ $(M+H)^+$ 304.2277, found 304.2288.

SCF$_F$: IR (neat, cm^{-1}) 1660, 1626, 1582, 1458, 1380, 1362; ^{1}H NMR (400 MHz, $C_4D_8O_2$) δ 0.869 (t, 3H, J = 7.3 Hz), 1.03 (s, 6H), 1.47-1.49 (m, 2H), 1.56 (q, 2H, J = 7.3 Hz), 1.60-1.66 (m, 2H), 1.72 (d, 3H, J = 0.7 Hz), 2.00 (d, 3H, J = 1.2 Hz), 2.03 (t, 2H, J = 7.2 Hz), 3.37 (t, 2H, J = 6.8 Hz), 6.02 (d, 1H, J = 9.3 Hz), 6.33 (d, 1H, J = 16.0 Hz), 6.80 (d, 1H, J = 16.0 Hz), 8.36 (d, 1H, J = 9.5 Hz); ^{13}C NMR (100 MHz, $C_4D_8O_2$) δ 12.08, 19.76, 20.49, 21.82, 24.80, 29.06, 29.16, 33.44, 34.75, 40.07, 64.07, 128.85, 130.33, 131.31, 136.97, 138.59, 142.58, 157.89.

SCF$_G$: IR (neat, cm^{-1}) 1718, 1646, 1612, 1462, 1384, 1228; ^{1}H NMR (400 MHz, $C_4D_8O_2$) δ 0.935 (t, 3H, J = 7.6 Hz), 1.06 (s, 6H), 1.46-1.49 (m, 2H), 1.61-1.69 (m, 4H), 1.77 (s, 3H), 2.06 (t, 2H, J = 6.4 Hz), 3.50 (t, 2H, J = 6.4 Hz), 3.67 (s, 3H), 6.01 (s, 1H), 7.38 (s, 2H), 8.07 (s, 1H); ^{13}C NMR (100 MHz, $C_4D_8O_2$) δ

12.10, 19.69, 21.83, 24.57, 29.14, 33.81, 34.74, 40.48, 51.35, 64.33, 121.47, 126.71, 132.68, 138.61, 138.66, 148.07, 161.74, 166.82; FAB HRMS m/z calcd. for $C_{19}H_{30}O_2N$ $(M+H)^+$ 304.2277, found 304.2264.

SCF$_H$: IR ($CHCl_3$, cm^{-1}) 1712, 1522, 1478, 1438, 1136, 1042; ^1H NMR (400 MHz, $C_4D_8O_2$) δ 0.891 (t, 3H, J = 7.6 Hz), 1.04 (s, 6H), 1.39-1.45 (m, 2H), 1.56-1.66 (m, 4H), 1.69 (s, 3H), 1.93 (t, 2H, J = 6.0 Hz), 2.10-2.14 (m, 2H), 2.56-2.60 (m, 2H), 3.47 (t, 2H, J = 6.8 Hz), 3.75 (s, 3H), 7.02 (d, 1H, J = 9.2 Hz), 8.30 (d, 1H, J = 9.2 Hz); ^{13}C NMR (100 MHz, $C_4D_8O_2$) δ 11.58, 12.01, 20.05, 24.39, 28.47, 28.83, 29.69, 33.32, 35.55, 40.47, 52.10, 64.74, 128.80, 136.56, 137.23, 140.68, 158.57, 168.20; FAB HRMS m/z calcd. for $C_{19}H_{32}O_2N$ $(M+H)^+$ 306.2433, found 306.2431.

SCF$_I$: IR (neat, cm^{-1}) 1724, 1458, 1440, 1386, 1258, 1168, 1092; ^1H NMR (400 MHz, $C_4D_8O_2$) δ 0.808 (d, 3H, J = 5.6 Hz), 0.85-0.91 (s, 3H, s, 3H, t, 3H), 1.12-1.27 (m, 1H), 1.41-1.67 (m, 8H), 1.72-1.75 (m, 1H), 3.40 (t, 2H, J = 6.8 Hz), 3.74 (s, 3H), 5.85 (dd, 1H, J = 15.9, 9.5 Hz), 6.33 (d, 1H, J = 15.6 Hz), 6.88 (d, 1H, J = 9.0 Hz), 8.35 (d, 1H, J = 9.3 Hz); ^{13}C NMR (100 MHz, $C_4D_8O_2$) δ 12.05, 20.76, 21.98, 22.53, 24.51 31.85, 32.26, 34.54, 36.16, 42.06, 52.14, 60.22, 64.60, 124.44, 134.93, 137.42, 142.28, 159.04, 167.64; FAB HRMS m/z calcd. for $C_{19}H_{32}O_2N$ $(M+H)^+$ 306.2433, found 306.2435.

2-Ethyl-5,5,8a-trimethyl-*trans*-3,4,4a,5,6,7,8,8a-octahydronaphthylmethanol. To cupper iodide (5.71 g, 30 mmol) in ether (40 mL) was added a 0.4 M solution of ethyl lithium in ether (150 mL, 60 mmol) prepared from ethyl bromide (37.3 mL, 500 mmol), lithium wire (8.6 g, 1.24 mol), and ether (200 mL) at -35 °C. The reaction mixture was stirred at -35 °C for 20 min and a solution of 2-diethoxyphosphoxy-1-methoxycarbonyl-5,5,8a-trimethyloctahydronaphthalene[25] (1.93 g, 5.0 mmol) in ether (10 mL) was added dropwise at the same temperature. After the mixture was stirred at -35 °C for an additional 15 min, saturated aqueous NH_4Cl solution was added at this temperature, and the resulting mixture was extracted with ether. The organic layers were combined, washed with saturated aqueous NH_4Cl solution, brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* to give the crude products. Column chromatography on silica gel (2 % ethyl acetate in hexane) gave the corresponding coupling product accompanied with the unseparable biproducts.

To a solution of the coupling reaction products in toluene (30 mL) was rapidly added a 1.0 M solution of diisobutyl aluminium hydride in hexane (40.0 mL, 40.0 mmol) at -78 °C. After the reaction mixture was stirred at this temperature for 15 min, 2 N HCl solution was slowly added, and the resulting mixture was extracted with ether. The organic layers were combined, washed with 2 N HCl solution, brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* to give the crude products. Column chromatography on silica gel (1 % ether in hexane) gave the corresponding alcohol (365 mg, 31 % for 2 steps): IR (neat, cm^{-1}) 3204, 2940, 1460, 1376, 1010; ^1H NMR (400 MHz, $CDCl_3$) δ 0.843 (s, 3H), 0.893 (s, 3H), 0.962 (s, 3H), 0.997 (t, 3H, J = 7.6 Hz), 1.11-1.21 (m, 2H), 1.24-1.31 (m, 1H), 1.37-1.46 (m, 2H), 1.49-1.72 (m, 3H), 1.88-1.91 (m, 1H), 1.97-2.20 (m, 4H), 4.01 (d, 1H, J = 11.6 Hz), 4.18 (d, 1H, J = 11.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.91, 18.83, 18.94, 19.06, 20.84, 21.59, 26.23, 30.54, 33.23, 36.86, 37.93, 41.63, 51.65, 57.86, 138.21, 140.42; FAB HRMS m/z calcd. for $C_{16}H_{27}$ $(M-H_2O+H)^+$ 219.2113, found 219.2126.

2-Ethyl-5,5,8a-trimethyl-*trans*-3,4,4a,5,6,7,8,8a-octahydronaphthylmethanesulfonyltoluene. To a ether (10 mL) solution of the alcohol obtained (260 mg, 1.10 mmol) and pyridine (0.076 mL, 0.944 mmol) was added phosphorous tribromide (0.134 mL, 1.42 mmol) at 0 °C. After the reaction mixture was stirred at room temperature for 12 min, H_2O was added, and the resulting mixture was extracted with ether. The organic

layers were combined, washed with H_2O, brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* to give the corresponding bromide.

To a DMF (8 mL) solution of the crude bromide was added *p*-toluenesulfinic acid sodium salt ($TolSO_2Na$) (421 mg, 2.36 mmol) at room temperature. After stirring at the same temperature for 1.5 h, the mixture was diluted with H_2O and was extracted with ethyl acetate. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* to give the crude products. Column chromatography on silica gel (2 % ethyl acetate in hexane) gave the corresponding sulfone (294 mg, 71 % for 2 steps): IR (neat, cm^{-1}) 2948, 1462, 1318, 1150, 1088, 714, 668, 582; ^1H NMR (400 MHz, $CDCl_3$) δ 0.845 (s, 3H), 0.898 (s, 3H), 0.953 (t, 3H, J = 7.6 Hz), 0.999 (s, 3H), 1.14-1.26 (m, 2H), 1.34-1.43 (m, 2H), 1.45-1.60 (m, 3H), 1.66-1.75 (m, 2H), 1.99-2.14 (m, 2H), 2.16-2.20 (m, 2H), 2.45 (s, 3H), 3.81 (d, 1H, J = 14.4 Hz), 3.99 (d, 1H, J = 14.4 Hz), 7.33-7.36 (m, 2H), 7.79-7.82 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.41, 18.61, 18.93, 20.64, 21.55, 21.65, 27.59, 30.13, 33.15, 33.34, 37.32, 37.81, 41.43, 50.71, 56.37, 127.61, 128.81, 129.70, 138.98, 142.96, 144.04; FAB HRMS m/z calcd. for $C_{23}H_{34}O_2SNa$ $(M+Na)^+$ 397.2177, found 397.2179.

3-[2,2-(2-Ethyl-5,5,8a-trimethyl-*trans*-3,4,4a,5,6,7,8,8a-octahydronaphthyl)-*p*-toluenesulfonylethyl]-2-trimethylsilylfuran. To a THF (7 mL) solution of the sulfone obtained above (290 mg, 0.774 mmol) and HMPA (1 mL) was added dropwise *n*-butyllithium (1.6 M solution in hexane, 0.581 mL, 0.929 mmol) at -78 °C. The reaction mixture was stirred at -78 °C for 13 min and a THF (3 mL) solution of chloride **5** (292 mg, 1.55 mmol) was added at the same temperature. After the mixture was gradually warmed to room temperature and stirred for an additional 25 min, H_2O was added, and the resulting mixture was extracted with ethyl acetate. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* to give the crude products. Column chromatography on silica gel (1 % ethyl acetate in hexane) gave the corresponding furan derivative as a mixture of stereoisomers: ^1H NMR (400 MHz, $CDCl_3$) δ 0.190 (s, 13/20×9H), 0.212 (s, 7/20×9H), 0.580 (s, 7/20×3H), 0.786 (s, 7/20×3H), 0.836 (s, 13/20×3H), 0.873 (s, 13/20×3H), 0.911 (s, 7/20×3H), 0.940-1.05 (m, 3H), 1.12 (s, 13/20×3H), 1.13-1.24 (m, 2H), 1.26-1.43 (m, 3H), 1.45-1.56 (m, 2H), 1.68-1.73 (m, 1H), 2.08-2.39 (m, 4H), 2.41 (s, 13/20×3H), 2.42 (s, 7/20×3H), 2.57-2.71 (m, 1H), 2.97-3.04 (m, 1H), 3.40-3.53 (m, 1H), 3.99-4.05 (m, 1H), 5.52 (d, 7/20×1H, J = 1.7 Hz), 5.66 (d, 13/20×1H, J = 1.7 Hz), 7.23-7.26 (m, 2H), 7.30 (d, 7/20×1H, J = 1.5 Hz), 7.31 (d, 13/20×1H, J = 1.5 Hz), 7.59 (td, 13/20×2H, J = 2.0, 8.3 Hz), 7.64 (td, 7/20×2H, J = 2.0, 8.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ -1.24, -1.20, 12.89, 13.02, 18.57, 18.99, 19.14, 19.25, 20.51, 21.49, 21.61, 21.66, 28.07, 28.68, 28.81, 29.00, 30.96, 31.12, 33.17, 33.36, 36.82, 36.97, 39.81, 39.94, 40.95, 41.22, 51.11, 51.46, 67.38, 67.52, 110.27, 111.00, 127.99, 128.24, 129.46, 129.55, 131.40, 132.02, 133.36, 134.39, 139.26, 139.91, 141.81, 142.15, 143.51, 143.70, 145.94, 146.27, 155.38, 155.48; FAB HRMS m/z calcd. for $C_{31}H_{47}O_3SiS$ $(M+H)^+$ 527.3016, found 527.2993.

4-Hydroxy-2-[2,2-(2-ethyl-5,5,8a-trimethyl-*trans*-3,4,4a,5,6,7,8,8a-octahydronaphthyl)-*p*-toluenesulfonylethyl]butenolide. To a solution of the furan derivative obtained in dichloromethane (7 mL) was added a catalytic amount of tetraphenylporphine (TPP), and the solution was cooled at -78 °C. The mixture was irradiated with a halogen lamp (Riko-HGA-300A) at -78 °C under an oxygen atmosphere, and the reaction

was monitored by TLC. After the starting material was consumed, the reaction mixture was flushed with argon. The solvent was removed *in vacuo* and the crude products were purified by column chromatography on silica gel (2 % ethyl acetate in hexane) to give the corresponding γ-hydroxybutenolide (31 % and 79 % of conversion yield from sulfone) as a mixture of stereoisomers: IR (disk, cm^{-1}) 3424, 2944, 1768, 1288, 1202, 1140, 1084, 1010; ^1H NMR (400 MHz, $CDCl_3$) δ 0.792-1.12 (m, 12H), 1.31-1.77 (m, 9H), 2.08-2.42 (m, 4H), 2.43 (s, 3H), 2.49-2.75 (m, 1H), 3.29-3.51 (m, 1H), 4.36 (dd, 7/20×1H, J = 4.8, 9.4 Hz), 4.43 (dd, 7/20×1H, J = 4.6, 9.5 Hz), 4.50 (dd, 3/20×1H, J = 3.7, 8.8 Hz), 4.56 (dd, 3/20×1H, J = 4.0, 9.6 Hz), 5.82 (s, 3/20×1H), 5.92 (s, 3/20×1H), 5.92 (s, 7/20×1H), 5.96 (s, 7/20×1H), 6.80 (s, 3/20×1H), 6.86 (s, 7/20×1H), 6.89 (s, 3/20×1H), 6.94 (s, 7/20×1H), 7.28-7.32 (m, 2H), 7.63-7.70 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.01, 13.16, 13.31, 14.14, 18.33, 18.38, 18.51, 18.93, 19.02, 19.12, 19.47, 19.79, 20.50, 20.58, 21.03, 21.56, 21.69, 28.63, 28.72, 28.78, 28.87, 28.96, 29.25, 29.62, 30.98, 31.06, 33.17, 33.36, 33.43, 36.56, 36.62, 36.99, 37.11, 40.04, 40.31, 40.49, 40.55, 41.02, 41.19, 41.27, 50.73, 50.84, 52.22, 52.45, 60.48, 62.50, 63.68, 63.88, 64.99, 96.61, 96.73, 128.12, 128.42, 128.66, 129.53, 129.60, 129.66, 129.71, 133.45, 133.59, 134.06, 134.36, 138.42, 138.98, 142.54, 142.73, 142.91, 144.34, 144.45, 144.55, 146.31, 146.68, 146.77, 170.56, 170.61, 170.72; FAB HRMS m/z calcd. for $C_{28}H_{39}O_5S$ $(M+H)^+$ 487.2518, found 487.2538.

Methyl (E,E)-4-oxo-2-[(2-ethyl-5,5,8a-trimethyl-*trans*-3,4,4a,5,6,7,8,8a-octahydronaphthyl)vinyl]but-2-enoate (E') and methyl (Z,E)-4-oxo-2-[(2-ethyl-5,5,8a-trimethyl-*trans*-3,4,4a,5,6,7,8,8a-octahydronaphthyl)vinyl]but-2-enoate. To a solution of the γ-hydroxybutenolide obtained above (50 mg, 0.103 mmol) in DMSO (2 mL) was added N,N'-diisopropylethylamine (0.054 mL, 0.308 mmol) at room temperature. The reaction mixture was stirred at room temperature for 3 h and methyl iodide (0.077 mL, 1.24 mmol) was added at the same temperature. After the mixture was stirred at room temperature for 20 min, saturated aqueous NH_4Cl solution was added, and the resulting mixture was extracted with ether. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* to give the corresponding aldehyde.

To a THF (1 mL) solution of crude aldehyde was added diazabicycloundecene (0.017 mL, 0.113 mmol) at 0 °C. After the reaction mixture was stirred at 0 °C for 10 min, saturated aqueous NH_4Cl solution was added, and the resulting mixture was extracted with ether. The organic layers were combined, washed with saturated aqueous NH_4Cl solution, brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* to give the crude products. Column chromatography on silica gel (5 % ethyl acetate in hexane) gave 1 : 9.3 mixture of E' and its (Z)-stereoisomer (20 mg, 57 % for 2 steps). These compounds were separated by column chromatography on silica gel (1 % ethyl acetate in hexane). Data for (Z)-stereoisomer: IR (neat, cm^{-1}) 2956, 1740, 1678, 1604, 1582, 1464, 1212, 1164; ^1H NMR (400 MHz, $CDCl_3$) δ 0.848 (s, 3H), 0.897 (s, 3H), 0.973 (t, 3H, J = 7.6 Hz), 1.04 (s, 3H), 1.11-1.19 (m, 3H), 1.39-1.50 (m, 3H), 1.56-1.64 (m, 2H), 1.70-1.74 (m, 1H), 1.97-2.09 (m, 2H), 2.14-2.16 (m, 2H), 3.94 (s, 3H), 6.05 (d, 1H, J = 7.6 Hz), 6.13 (d, 1H, J = 16.4 Hz), 6.59 (d, 1H, J = 16.1 Hz), 9.76 (d, 1H, J = 7.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.84, 18.54, 18.94, 20.55, 21.56, 27.70, 30.72, 33.17, 33.35, 28.27, 38.36, 41.56, 51.18, 52.62, 127.85, 127.87, 137.54, 140.19, 140.24, 150.16, 166.70, 190.75. Data for (E)-stereoisomer E': IR ($CHCl_3$, cm^{-1}) 2973, 1726, 1672, 1522, 1424; ^1H NMR (400 MHz, $CDCl_3$) δ 0.854 (s, 3H), 0.904 (s, 3H), 1.00 (t, 3H, J = 7.4 Hz), 1.05 (s, 3H), 1.13-1.73 (m, 9H), 2.03-2.18 (m, 4H), 3.86 (s, 3H), 6.55 (s, 2H), 6.62 (d, 1H, J = 7.6 Hz), 10.05 (d, 1H, J = 7.3 Hz); ^{13}C

NMR (100 MHz, $CDCl_3$) δ 13.98, 18.57, 18.97, 20.55, 21.56, 27.84, 30.63, 33.17, 33.35, 38.17, 38.39, 41.56, 51.15, 52.76, 122.93, 131.20, 137.15, 140.72, 142.08, 146.48, 167.13, 191.54; FAB HRMS m/z calcd. for $C_{22}H_{33}O_3$ $(M+H)^+$ 345.2430, found 345.2423.

Preparation of bovine pancreatic PLA_2 modified by the derivatives A-I[9c]: To a 6.10×10^{-5} M solution of bovine pancreatic PLA_2 (10 μL, ionic strength μ = 0.2) were added a 50 mM Tris/HCl buffer solution (100 μL, pH 8.0, μ = 0.2) and a 6.10×10^{-3} M solution of the derivatives **A-I** in 1,4-dioxane (10 μL), and the mixture was incubated at 40 °C for an appropriate time interval (15, 30, 45, 60, and 90 min). For the control experiment, the same volume (10 μL) of dioxane was added instead of a dioxane solution of the derivative. The final concentrations of PLA_2, the derivatives, and dioxane in the incubation mixture were 4 μM, 0.4 mM, and 8.0 %, respectively, and the ionic strength was adjusted to 0.2 with NaCl.

Assay method: PLA_2 activity was measured by the pH-statt assay method using a system consisting of Radiometer PHM84 standard pH meter, a TTT80 titrator and an ABU80 autoburette. To a 1 mM solution of the anionic micelles of the substrates (1 mL) prepared from 1,2-didodecanoyl-*sn*-glycero-3-phosphocholine and sodium cholate in the presence of $CaCl_2$ (The concentrations of 1,2-didodecanoyl-*sn*-glycero-3-phospho-choline, cholic acid, and $CaCl_2$ were 1 mM, 5 mM, and 10 mM, respectively, and the ionic strength was adjusted to 0.2 with NaCl.) was added a solution of the modified PLA_2 (20 μL) taken from the incubation mixture, and the fatty acids released from glycerophospholipids were titrated with a 30 mM NaOH solution to adjust to pH 7.0 and at 25 °C under an argon atmosphere.

Amino acid analysis of the PLA_2 modified by the derivatives A-I[9c]: To a 6.10×10^{-5} M solution of bovine pancreatic PLA_2 (80 μL, ionic strength μ = 0.2) were added a 50 mM Tris/HCl buffer solution (800 μL, pH 8.0, μ = 0.2) and a 6.10×10^{-3} M solution of the derivative **A-I** in 1,4-dioxane (80 μL), and the mixture was incubated at 40 °C for an appropriate time interval (15, 30, 45, 60, and 90 min). For the control experiment, the same volume (80 μL) of dioxane was added instead of a dioxane solution of the derivative. The final concentrations of PLA_2, the derivatives, and dioxane in the incubation mixture were 4 μM, 0.4 mM, and 8.0 %, respectively, and the ionic strength was adjusted to 0.2 with NaCl. To 1 % acetic acid (300 μL) was added a solution of the modified PLA_2 (200 μL) taken from the incubation mixture, and the resulting mixture was immediately desalted with NAP-5 column (Pharmacia LKB Biotechnology) equilibrated with 1 % acetic acid. The eluted protein fraction was concentrated, and the residue was hydrolyzed with 5.7 M HCl and 0.2 % phenol vapor in a sealed tube *in vacuo* at 110 °C for 24 h. After evaporation of the solvents, the hydrolysates were analyzed using a Hitachi L-8500 amino acid analyzer.

Preparation and isolation of the PLA_2 modified by the derivative E[9c]: To a 4.50×10^{-5} M Tris/HCl buffer solution of bovine pancreatic PLA_2 (2.5 mL, pH 8.0 and ionic strength μ = 0.2) was added a 4.50×10^{-2} M solution of the derivative **E** in 1,4-dioxane (25 μL). The final concentrations of PLA_2, derivative **E**, and dioxane were 4.46×10^{-5} M, 4.46×10^{-4} M, and 0.1 %, respectively. The reaction mixture was incubated at 40 °C for 60 min, then immediately passed through a Sephadex G-25 column (1.6 cm×23 cm) pre-equilibrated with

50 mM acetate buffer (pH 5.0). The PLA_2 modified by the derivative **E** and intact PLA_2 were separated by the following method. The eluted protein fractions by a Sephadex G-25 column were dialyzed for 24 h against 50 mM acetate buffer (pH 5.0) containing 8 M urea to give the 8 M urea solution of the mixture of the modified and intact PLA_2 (1.78 mg). These proteins were fractionated on a Mono-S column (0.5 cm×5 cm) pre-equilibrated with the buffer used for the dialysis described above, and eluted with the same buffer containing a linear concentration gradient of NaCl from 0 to 0.1 M. The separated protein fractions were respectively dialyzed for two days against 1 % acetic acid, and concentrated *in vacuo* at 50 °C to give 22.6 nmol of intact PLA_2 and 8.80 nmol of the PLA_2 modified by one molecule of the derivative **E**; MALDI-TOF-MS m/z calcd. for PLA_2 plus one dihydropyridine derivative of **E** $(M+H)^+$ 14,095, found 14,098.

Cleavage of the seven disulphide bonds and S-pyridylethylation of the resulting fourteen systeine residues in bovine PLA_2: S-pyridylethyl derivative of the PLA_2 modified by the derivative **E** (PE-PLA_2) was prepared according to the method of Cavins and Friedman.[23] The PLA_2 modified by the derivative **E** (4.6 nmol) was dissolved in a 50 mM Tris/HCl buffer solution (100 μL, pH 8.0) containing 6 M guanidine/HCl and 10 mM EDTA at 50 °C for 1 h, and reduced with dithiothreitol(0.248 mg, 350 equivalent) at 50 °C for 2.5 h. To the reaction mixture was added 4-vinylpyridine (0.508 μL, 1050 equivalent) and reacted at room temperature for 2.5 h with the exclusion of light. The mixture was desalted by dialysis against 1 % acetic acid and concentrated at 50 °C *in vacuo* to give S-pyridylethyl derivative of the modified PLA_2 (1.59 nmol); MALDI-TOF-MS m/z calcd. for S-pyridylethyl PLA_2 plus one dihydropyridine derivative of **E** $(M+H)^+$ 15,581, found 15,580.

Digestion: For lysyl endopeptidase digestion, the modified PLA_2 (18 μg, 1.3 nmol) was dissolved in a 50 mM Tris/HCl buffer solution (30 μL, pH 9.0) containing 8 M urea at 37 °C for 3 h, then lysyl endopeptidase in a 50 mM Tris/HCl buffer solution (0.18 μg, 30 μL, pH 9.0) was added. After the reaction mixture was incubated at 37 °C for 12 h, it was directly subjected to MALDI-TOF-MS analyses. For Asp-N digestion, the modified PLA_2 (14 μg, 1.0 nmol) was dissolved in a 50 mM Tris/HCl buffer solution (40 μL, pH 7.5) containing 4 M urea at 37 °C for 3 h, then Asp-N in a 50 mM Tris/HCl buffer solution (0.14 μg, 40 μL, pH 7.5) was added. After the reaction mixture was incubated at 37 °C for 6 h, it was directly subjected to MALDI-TOF-MS analyses.

Mass Spectrometry

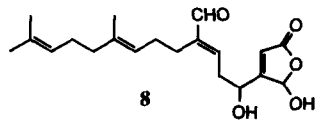
Mass spectra were acquired on a Voyager Elite MALDI-time-of-fight mass spectrometer (PerSeptive Biosystems, Framingham, MA, USA) equipped with delayed extraction source and 337 nm pulsed nitrogen laser. Linear (for PLA_2) and reflector (for peptide fragments) were utilized with 20 kV acceleration voltage. For matrix, sinapinic acid and CHCA (α-cyano-4-hydroxy cinnamic acid) (Aldrich) were dissolved in a 30 % acetonitrile solution (0.1 % TFA) and 50 % acetonitrile solution (0.1 % TFA) at 10 g / L, respectively. Samples were dissolved in 50 % acetonitrile (0.1 % TFA) to have a concentration of about 10^{-5} M, and 0.5 μL of the sample were mixed with 1 μL of matrix. Prepared sample solutions (0.5 μL) were deposited on the sample plate and air dried.

References and Notes

1. Dennis, E. A., *The Enzymes* **16**, p. 307, Boyer, P. D. ed., Academic Press, NewYork (1983).
2. Pace-Asciak, C. R.; Smith, W. L., *The Enzymes* **16**, p. 543, Boyer, P.D. ed., Academic Press, New York (1983).
3. (a) Waite, M. in "Handbook of Lipid Research, Volume 5: The Phospholipases." Ed. by Hanahan, D. J., Plenum Press, New York, **1987**, p. 111. (b) Scott, D. L.; Sigler, P. B. *Advances in Protein Chemistry*; Scumaker, V. N., Ed.; Academic Press: San Diego, **1994**; Vol. 45, p. 53.
4. Potts, B. C. M.; Faulkner, D. J.; Jacobs, R. S. *J. Nat. Prods.*, **1992**, *55*, 1701.
5. (a) de Silva, D. E.; Scheuer, P. J. *Tetrahedron Lett.*, **1980**, *21*, 1611. (b) de Silva, E. D.; Scheuer, P. J. *Tetrahedron Lett.*, **1981**, *22*, 3147.
6. (a) Cimino,G.; de Stefano, S.; Minale, L. *Experientia*, **1974**, *30*, 846. (b) Cimino, G.; Sodano, G.; Spinella, A. *Tetrahedron*, **1987**, *43*, 5401. (c) de Carvalho, M.S.; Jacobs, R.S. *Biochem. Pharmacol.*, **1991**, *42*, 1621.
7. Inhibitory mechanism toward bee venom PLA_2; (a) Burley, E. S.; Smith, B.; Cutter, G.; Ahlem, J. K.; Jacobs, R. S. *Pharmacologist*, **1982**, *24*, 138. (b) de Freitas, J. C.; Blankemeier, L. A.; Jacobs, R. S. *Experientia*, **1984**, *40*, 864. (c) Jacobs, R. S.; Culver, P.; Langdon, R.; O'Brien, T.; White, S. *Tetrahedron*, **1985**, *41*, 981. (d) Glaser, K. B.; Jacobs, R. S. *Biochem. Pharmacol.*, **1986**, *35*, 449. (e) Bennett, C.F.; Mong, S.; Clarke, M.A.; Kruse, L.I.; Crooke, S.T. *Biochem. Pharm.*, **1987**, *36*, 733. (f) Kernan, M. R.; Faulkner, D. J.; Jacobs, R. S. *J. Org. Chem.*, **1987**, *52*, 3081. (g) Albizati, K. F.; Holman, T.; Faulkner, D. J.; Glaser K. B.; Jacobs, R. S. *Experientia*, **1987**, *43*, 949. (h) Kernan, M. R.; Faulkner, D. J. *J. Org. Chem.*, **1988**, *53*, 2773. (i) Glaser, K. B.; Vedvick, T. S.; Jacobs, R. S. *Biochem. Pharmacol.*, **1988**, *37*, 3639. (j) Kernan, M. R.; Faulkner, D. J.; Parkanyi, L.; Clardy, J.; de Carvalho, M. S.; Jacobs, R. S. *Experientia*, **1989**, *45*, 388. (k) Glaser, K. B.; de Carvalho, M. S.; Jacobs, R. S.; Kernan, M. R.; Faulkner, D. J. *Mol. Pharmacol.*, **1989**, *36*, 782. (l) de Carvalho, M. S.; Jacobs, R. S. *Biochem. Pharmacol.*, **1991**, *42*, 1621. (m) Potts, B. C. M.; Capon, R. J.; Faulkner, D. J. *J. Org. Chem.*, **1992**, *57*, 2965. (n) Potts, B. C. M.; Faulkner, D. J.; de Carvalho, M. S.; Jacobs, R. S. *J. Am. Chem. Soc.*, **1992**, *114*, 5093. (o) Ortiz, A. R.; Pisabarro, M. T.; Gago, F. *J. Med. Chem.* **1993**, *36*, 1866.
8. Inhibitory mechanism toward cobra venom PLA_2; (a) Lombardo, D.; Dennis, E. A. *J. Biol. Chem.*, **1985**, *260*, 7234. (b) Deems, R. A.; Lombardo, D.; Morgan, B. P.; Mihelich, E. D.; Dennis, E. A. *Biochim. Biophys. Acta*, **1987**, *917*, 258. (c) Reynolds, L. J.; Morgan, B. P.; Hite, G. A.; Mihelich, E. D.; Dennis, E. A. *J. Am. Chem. Soc.*, **1988**, *110*, 5172. (d) Reynolds, E. J.; Mihelich, E. D.; Dennis, E. A. *J. Biol. Chem.*, **1991**, *266*, 16512. (e) Reynolds, L. J.; Michelich, E. D.; Dennis, E. A. *J. Biol. Chem.*, **1992**, *266*, 16512. (f) Plesniak, L. A.; Boegeman, S. C.; Segelke, B. W.; Dennis, E. A. *Biochemistry*, **1993**, *32*, 5009. (g) Dennis, E. A. *J. Biol. Chem.*, **1994**, *269*, 13057. (h) Bianco, I. D.; Kelley, M. J.; Crowl, R. M.; Dennis, E. A. *Biochim. Biophys. Acta*, **1995**, *1250*, 197.
9. (a) Katsumura, S.; Han, Q.; Kadono, H.; Fujiwara, S.; Isoe, S.; Fujii, S.; Nishimura, H.; Ikeda, K. *Bioorg. Med. Chem. Lett.*, **1992**, *2*, 1263. (b) Katsumura, S.; Han, Q.; Fujiwara, S.; Isoe, S.; Nishimura, H.; Inoue, S.; Ikeda, K. *Bioorg. Med. Chem. Lett.*, **1992**, *2*, 1267. (c) Fujii, S.; Tahara, Y.; Toyomoto, M.; Hada, S.; Nishimura, H.; Inoue, S.; Ikeda, K.; Inagaki, Y.; Katsumura, S.; Samejima,

Y.; Omori-Satoh, T.; Takasaki, C.; Hayashi, K. *Biochem. J.*, **1995**, *308*, 297.

10. We had obtained the following unpublished results. C20 analog **8**[9c] showed nearly same extent of inhibitory activity toward bovine PLA_2, and the reaction of **8** with 1,4-butanediamine and 1,3-propanediamine gave a complex mixture, respectively, while the reaction of **8** with 1,6-hexanediamine and 1,5-pentanediamine gave red colored compounds, which were insoluble to every solvent except mineral acids. These results suggest that manoalide may react with two different lysine residues as the proposal of Jacobs' group[4,7i,n] to produce insoluble polymerized compounds.



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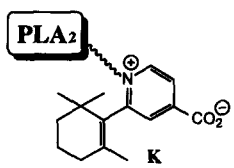
11. (a) Dijkstra, B. W.; Brenth, J.; Kalk, H. H. *Nature*, **1981**, *289*, 604. (b) Dijkstra, B. W.; Kalk, K. H.; Hol, W. G. J.; Drenth, J. *J. Mol. Biol.*, **1981**, *147*, 93.
12. Ito, M.; Katsuta, Y.; Yamano, Y.; Tsukida, K. *J. Chem. Soc. Perkin Trans.l*, **1993**, 987.
13. Tanaka, K.; Kamatani, M.; Mori, H.; Fujii, S.; Ikeda, K.; Hisada, M.; Itagaki, Y.; Katsumura, S. *Tetrahedron Lett.*, **1998**, *39*, 1185.
14. Negishi, E.; King, A.O.; Klima, W.L. *J. Org. Chem.*, **1980**, *45*, 2526.
15. Sato,F.; Ishikawa,H.; Watanabe,H.; Miyake,T.; Sato,M. *J. Chem. Soc. Chem. Commun.*, **1981**, 718.
16. Kocienski, P.J.; Love, C.J.; Whitby, R.J.; Costello, G.; Roberts, D.A. *Tetrahedron*, **1989**, *45*, 3839.
17. Katsumura, S.; Hori, K.; Fujiwara, S.; Isoe, S. *Tetrahedron Lett.*, **1985**, *26*, 4625.
18. Stepwise formation from **7**; methylesterification (MeI, i-Pr_2NEt / DMSO; 88% yield) and then elimination of sulfone (DBU, $Pd(Ph_3P)_4$/ DMSO; 94% yield) gave 2 : 5 mixtures of **A** and **B**.
19. When the concentration of these compounds was lowered, **A** and **E**, which have cyclic hydrophobic moieties, exibited stronger inhibitory activities than the linear derivatives **C** and **D**.
20. The dihydropyridine compounds obtained from the reaction of **A**, **C**, **D**, and **E** with *n*-propylamine were unstable in acid. We then monitored these reactions by NMR in dioxane-d_8 or C_6D_6.
21. (a) de Lera, A. R.; Reischl, W.; Okamura, W. H. *J. Am. Chem. Soc.*, **1989**, *111*, 4051. (b) Maynard, D. F.; Okamura, W. H. *J. Org. Chem.*, **1995**, *60*, 1763, and references cited therein.
22. Ren, R. X.-F.; Sakai, N.; Nakanishi, K. *J. Am. Chem. Soc.*, **1997**, *119*, 3619.
23. Cavins, J. F.; Friedman, M. *Anal. Biochem.*, **1970**, *35*, 489.25.
24. The results by MALDI-TOF-MS and FAB-MS analyses suggested that the 1,2-dihydropyridine derivatives might be aromatized and hydrolyzed to give the zwitterion such as **K** accompanied with loss of CH_4 (16 mass unit). The decomposition of the dihydropyridine derivatives in the modified PLA_2 might occur during the digestion by Lys-C and Asp-N, or during the MS analysis of the fragments obtained, since S-pyridylethyl derivative of PLA_2 was analyzed by MALDI-TOF-MS (see experimental section).



K

25. Hashimoto, S.; Sonegawa, M.; Sakata, S.; Ikegami, S. *J. Chem. Soc. Commun.*, **1987**, 24.
26. Noel, J. P.; Bingman, C. A.; Deng, T.; Dupureur, C. M.; Hamilton, K. J.; Jiang, R.-T.; Kwak, J.-G.; Sekharudu, C.; Sundaralingam, M.; Tsai, M.-D. *Biochemistry*, **1991**, *30*, 11801.
27. Barrero,A.F.; A-Manganeda,E.; Altrarejos,J.; Salido,S.; Ramos,J.M.*Tetrahedron Lett.*,**1994**,*35*, 2945.
28. (a) Goldsmith, D.; Liotta, D.; Saindane, M.; Waykole, L.; Bowen, P. *Tetrahedron Lett.* **1983**, *24*, 5835. (b) Tanis, S. P.; Head, D. B. *Tetrahedron Lett.* **1984**, *25*, 4451.
29. Torrado, A.; Iglesias, B.; Lopez, S.; de Lera, A. R. *Tetrahedron*, **1995**, *51*, 2435.