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Total Synthesis Confirms the Molecular Structure Proposed for Oxidized Levuglandin D₂

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Abstract

Levuglandin (LG)D₂ and LGE₂ are γ -ketoaldehyde levulinaldehyde derivatives with prostanoid side chains produced by spontaneous rearrangement of the endoperoxide intermediate PGH₂ in the biosynthesis of prostaglandins. Covalent adduction of LGs with the amyloid peptide A β ₁₋₄₂ promotes formation of the type of oligomers that have been associated with neurotoxicity and are a pathologic hallmark of Alzheimer's disease. Within 1 min of their generation during the production of PGH₂ by cyclooxygenation of arachidonic acid, LGs are sequestered by covalent adduction to proteins. In view of this high proclivity for covalent adduction, it is understandable that free LGs have never been detected in vivo. Recently a catabolite, believed to be an oxidized derivative of LGD₂ (ox-LGD₂), a levulinic acid hydroxylactone with prostanoid side chains, was isolated from the red alga *Gracilaria edulis* and detected in mouse tissues and in the lysate of phorbol-12-myristate-13-acetate (PMA)-treated THP-1 cells incubated with arachidonic acid. Such oxidative catabolism of LGD₂ is remarkable because it must be outstandingly efficient to prevail over adduction with proteins and because it requires a unique dehydrogenation. We now report a concise total synthesis that confirms the molecular structure proposed for ox-LGD₂. The synthesis also produces ox-LGE₂ that readily undergoes allylic rearrangement to Δ^6 -ox-LGE₂.

TOC image

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Supporting Information

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Experimental procedures, analytical data (¹H NMR, ¹³C NMR, MS, for all new compounds, additional reaction optimization tables (PDF)

The authors declare no competing financial interest.

Levuglandins (LGs) are secoprostaglandins, levulinaldehyde derivatives with prostanoid side chains, that are generated through cyclooxygenase (COX)-induced oxidation of arachidonic acid followed by spontaneous non-enzymatic rearrangement, involving a novel intramolecular 1,2-hydride shift, of an endoperoxide intermediate PGH₂ (Scheme 1).¹ Hydride (highlighted in red in Scheme 1) migration from C-11 to an incipient ketone methyl group produces LGD₂ while migration from C-9 produces LGE₂. LGs can also be generated through free radical-induced cyclooxygenation of arachidonate esterified to lyso phospholipids.² Owing to their reactive γ -ketoaldehyde functional arrays, LGs avidly bind with the primary amino groups of protein lysyl residues,³ DNA⁴ and ethanolamine phospholipids.⁵ Within 1 minute of their generation during the production of PGH₂ by cyclooxygenation of arachidonic acid, LGs are sequestered by covalent adduction to proteins.⁶ This covalent adduction as well as LG-induced cross-linking of proteins⁷ and DNA⁴ interferes with protein function⁸ and contributes to pathological processes, including brain tissue damage,⁹ tubulin malfunction,¹⁰ interference with the control of expression,¹¹ Alzheimer's disease (AD),^{12–15} age-related macular degeneration,^{16–18} atherosclerosis,^{19–22} end-stage renal disease,²³ hyperoxia,⁵ myocardial infarction²⁴ and sepsis.²⁵

Recently, a natural product, presumed to be ox-LGD₂ (**1**, Scheme 1), was isolated from the red alga *Gracilaria edulis* and was also detected in mouse tissues and in the lysate of phorbol-12-myristate-13-acetate treated THP-1 cells incubated with arachidonic acid.²⁶ Ox-LGD₂ is a γ -hydroxy-butenolide with prostanoid side chains that is presumably generated through oxidation of LGs (Scheme 1). In view of the exceptionally high proclivity for LGs to form covalent adducts with primary amino groups in biomolecules, a competing alternative fate, i.e., oxidation to ox-LGs *in vivo* was unexpected. Thus, within 1 min of incubation with bovine serum albumin, 10 equivalents of LGE₂ bind to the protein.³ Incubation of arachidonic acid with PGH-synthase-2 results in binding of 12 equivalents within 1 min.⁶ Furthermore, the presumed conversion of LGs to ox-LGs involves an unusual dehydrogenation that introduces the butenolide C=C bond. Because ox-LGD₂ lacks a reactive γ -ketoaldehyde functional array it is expected to be less prone than LGD₂ toward formation of covalent adducts with biomolecules. Consequently, the conversion of LGs into ox-LGs might serve as a biological detoxification process that can prevent the pathological consequences associated with the generation of LGs. To confirm its molecular structure, and as a prelude to investigating its biological functions and biosynthesis, we developed a concise unambiguous total synthesis of ox-LGD₂.

RESULTS AND DISCUSSION

Retrosynthetic Analysis

Because the nonenzymatic rearrangement of PGH₂ to LGD₂ is accompanied by the formation of LGE₂ that should lead to the production of ox-LGE₂ (Scheme 1), our synthetic strategy (Scheme 2) was designed to also deliver this structural isomer. Although our synthesis did produce a mixture of regioisomeric ox-LGs, owing to a proclivity toward rearrangement of the 5,6-C=C bond, only the rearrangement product ⁶-ox-LGE₂ is likely to be isolated from biological sources. The synthetic ox-LGD₂ proved identical to the compound isolated from biological sources.

A three component coupling strategy for preparing tri and tetrasubstituted olefins²⁷ was adopted as a key step for the total synthesis of ox-LGs (Scheme 2). Thus, conjugate addition of a nucleophilic lower side chain fragment, a higher-order cyanocuprate derived from a vinylstannane **5**, to di-tert-butyl acetylenedicarboxylate (**6**), and trapping of the resulting vinyl nucleophile product with an electrophilic upper side chain fragment, an allylic bromide **7**, was expected to deliver an anhydride **3** to which addition of a one-carbon nucleophile would deliver both ox-LGD₂ (**1**) and ox-LGE₂ (**2**). The mild conditions required to convert an intermediate di-tert-butyl ester **4** into a cyclic anhydride **3** were expected to be compatible with a sensitive allylic silyl ether functionality. A di-tert-butyl(methyl)silyl (DTBMS) ester in **3** was expected to be resistant to the reaction with methyllithium that is required for introduction of the final skeletal carbon.

Synthesis of the Carboxylic Acid Upper Side Chain

The upper side chain synthon, di-tert-butyl(methyl)silyl (*Z*)-7-bromohept-5-enoate (**7**), was prepared from tetrahydrofuran and propargyl alcohol. This new synthetic strategy for preparing **7** is an improvement over a previous synthesis involving alkylation of propargyl THP ether with 1-bromo-3-chloropropane, conversion of the bromide to a nitrile with cyanide in hot DMSO, hydrolysis of the nitrile, silylation, removal of the THP protecting group, catalytic partial hydrogenation and bromodehydroxylation.²⁸ The use of P-2 nickel boride catalyst²⁹ to selectively reduce the triple bond in **9** (Scheme 3) provided better selectivity than Lindlar's catalyst used in a previous synthesis of **7**.²⁸ The lower side chain vinyl nucleophile precursor **5** is readily available from 1-octyn-3-ol.³⁰ The use of a DTBMS ester was expected to prevent addition of MeLi to the terminal carboxyl during selective addition to the anhydride carbonyl in **3** and selective removal of tert-butyl protecting groups in **4**, and yet allow removal of the silyl protecting groups in the final step of the synthesis.

Three-component Coupling

Stereocontrolled syn-addition of organocopper reagents $\text{RCu}(\text{Me}_2\text{S})\text{MgBr}_2$ to acetylene dicarboxylates had been employed to prepare various tri and tetra-substituted maleates and their corresponding maleic anhydrides.^{31,32} In contrast with *n*-butylcuprate, yields were poor for the vinyl organocopper reagent generated from the lower side chain precursor **5** (Scheme 4, Table S1). Three-component coupling of the vinyltin **5** with acetylene **6** and the upper side chain precursor **7** delivered only a 5% of the yield of the desired product **4**.

A model study with a series of lower- and higher-order vinyl cuprates ($n = 1$ or 2 , $m = 1$ or 2), anionic ligands and reaction conditions revealed that several cyanocuprate reagents (Table S2) provided a nearly five- fold improvement in the yield of **14** compared to that with the organocopper reagent $\text{RCu}(\text{Me}_2\text{S})\text{MgBr}_2$ employed previously (Scheme 5). A comparison of reagents (Table S3) identified a divinyl cyanocuprate intermediate **15** ($n = 2$, $X = \text{CN}$) as the reagent of choice for the production of **4** by conjunction with the upper side chain allylic bromide **7**. The corresponding vinyl cyanocuprate **15** ($n = 1$, $X = \text{CN}$) gave a substantially lower yield of three-component coupling (14% versus 53%) and the corresponding vinyl bromocuprate **15** ($n = 1$, $X = \text{Br}$) was even worse (5%). However, for all of the cuprates, there was no preference for the desired $\text{S}_{\text{N}}2$ product **4** versus the unwanted $\text{S}_{\text{N}}2'$ product **4'** (Scheme 4). Fortunately, these products of α and γ substitution were

readily separable by semi-preparative HPLC (Figure S1), using CH₃CN and iso-propanol as eluent.

Generation of the ox-LG Hydroxybutenolide

Our strategy to complete the carbon skeleton of ox-LGs envisioned selective addition of a methyl nucleophile to one of the ester carbonyls in **4**. Conversion of the *cis* bis tert-butoxycarbonyl array in **4** into an anhydride **3** was envisioned as a strategy to selectively enhance the reactivity of the maleate carbonyls. However, this key manipulation would require selective dealkylation of the tert-butyl esters without desilylation. The development of methodology to accomplish these goals and a concise total synthesis of ox-LGs (Scheme 4) was enabled by two model studies. One model study (Scheme S1) confirmed the challenge of achieving the requisite selectivity. Treatment of di-tert-butyl ester **14** with formic acid simultaneously removed the tert-butyl groups and promoted cyclization to an anhydride, but also cleaved the TBDMS group to deliver anhydride **16**. Treatment of **14** with ZnBr₂ promoted transesterification to deliver lactone **17**. Ultimately, a mild deprotection method (silica gel/toluene/100 °C) selectively cleaved the tert-butyl esters in **14** without removing the silyl ether delivering anhydride **18**.³³ Similar treatment of di-tert-butyl ester **4** (Scheme 4) selectively cleaved the tert-butyl esters and provided the desired cyclic anhydride intermediate **3** without removal of the silyl ether or ester protecting groups.

As a model system for the selective monomethylation of the anhydride **3** (Scheme 4), dimethylmaleic anhydride (**19**), was treated with various methyl organometallics in the presence of the DTBMS ester **22** (Table S4). As planned, under all conditions examined, the DTBMS ester exhibited no reactivity. MeMgBr, MeLi and MeCeCl₂ all generated the desired monomethylation product **20** and an undesired geminal dimethylation product **21**. MeLi was the most selective at –78 °C. Nearly complete selectivity and excellent yield of the desired mono addition product **20** were achieved at –94 °C. Based on the integrated ¹H NMR peak areas for resonances centered at δ_H 6.50 and 6.32 (Figure S2), corresponding to H-13 in **1p** and **2p** respectively (Scheme 4), reaction of anhydride **3** with MeLi under these optimized reaction conditions delivered a 1:6 mixture of the silyl protected ox-LG derivatives **1p** and **2p** respectively. This unfortunate selectivity disfavoring the carbon skeleton required for ox-LGD₂, the primary target of our total synthesis, was counter balanced by the serendipitous instability of ox-LGE₂ that facilitated isolation of ox-LGD₂ from the mixture (*vide infra*).

The structures assigned to the minor and major products **1p** and **2p** were indicated by their 2-D NMR spectra. An HSQC spectrum (Figure S4) confirmed assignments of the proton resonances in these isomers. In the HMBC spectrum of the mixture of isomers (Figure S5), strong long-range C-8–H-13 and C-9–H-13 correlations (Figure 1) indicate that the H-13 peak belongs to isomer **2p** (precursor of ox-LGE₂). A C-11'–H-13' correlation indicates that the H-13' peak corresponds to the precursor **1p** of ox-LGD₂. The same conclusion is also supported by a NOESY spectrum of these isomers (Figure S6) that exhibits a strong correlation (Figure 1) between the H₃-10 and H₂-7 resonances assigned to **2p** whereas the resonances assigned to compound **1p** show H-10'–H-13' correlation.

Generation of Ox-LGD₂ by Removal of the Silyl Ether and Silyl Ester Protecting Groups

The γ -isomers **1p'** and **2p'** were generated from **4'** (Scheme 6) by the same chemistry employed to generate **1p** and **2p** from **4** (Scheme 4). Two reagents, Bu₄NF in THF or 40% HF in CH₃CN, both provided high isolated yields of **1'** and **2'** (81% or 100%) from a mixture of **1p'** and **2p'**.

However, these two reagents gave very different results when applied to a mixture of the silyl protected isomers **1p** and **2p**. Treatment of the mixture of silylated isomers with Bu₄NF gave only 18% yield of desilylated product (Table 1). Notably, isomerically pure ox-LGD₂ was the only product isolated. Apparently, ox-LGE₂ was selectively destroyed under these reaction conditions. The ¹H NMR spectrum of the ox-LGD₂ generated by our total synthesis agreed with that reported previously for the ox-LGD₂ extracted from *Gracilaria edulis* algae (Figure 2).

Generation and Rearrangement of Ox-LGE₂

Treatment of the mixture of silylated isomers with HF gave a much better yield (70%) of desilylated products that consisted of a 1:6 mixture of ox-LGD₂ (from **1p**) and two diastereomers of ox-LGE₂ (from **2p**), the major isomer showing a doublet in the ¹H NMR spectrum at δ_{H} 6.32 (ox-LGE₂ (**2a**)) and a minor diastereomer evidenced by small shoulders on the major doublet (ox-LGE₂ (**2b**)). The presence of the minor diastereomer was confirmed after HPLC isolation (*vide infra*). The doublet, assigned to the major ox-LGE₂ diastereomer, showed a first-order AMX ($\nu/J = 15$) spin system in contrast to the peaks assigned to ox-LGD₂ that exhibit a second-order ABX spin system ($\nu/J \approx 2$) between H-13, H-14 and H-15 (Figure 4). Two-dimensional NMR experiments, including HMBC, COSY and HSQC (Figures S7-S9), further confirmed the structure of the major ox-LGE₂ diastereomer. The COSY spectrum (Figure S7) and the ¹H NMR spectrum showed that the major ox-LGE₂ isomer has a structure with connectivity nearly identical with ox-LGD₂. This conclusion was bolstered by the HSQC spectrum (Figure S8) that enabled an accurate assignment of the ¹³C NMR resonances. However, an HMBC experiment (Figure S9) established that the position of C-10 methyl group in the major product corresponded to that of ox-LGE₂, the regioisomer of ox-LGD₂. Similar to observations made in the structural characterization of the precursor **2p**, a strong HMBC correlation (Figure 3, Figure S9) between the C-9 carbonyl and the C-13 vinyl carbon of the lower side chain as well as C-8–H-13 and C-8–H-14 correlations indicated that the structure of the major product corresponds to ox-LGE₂, which has a methyl group adjacent to the carboxyl side chain rather than adjacent to the vinyl side chain as in ox-LGD₂.

Upon standing in CD₃CN solution at room temperature, the ox-LGE₂ diastereomers in the mixture of ox-LGs, which was generated through desilylation with HF, decomposed. The disappearance of the ox-LGE₂ diastereomers from the mixture of ox-LGD₂ and ox-LGE₂ diastereomers was accompanied by the appearance of a new product. The *cis* 5-6 C=C bond of the ox-LGE₂ diastereomers migrated into conjugation to give ⁶-ox-LGE₂ while ox-LGD₂ in the mixture remained unchanged.

The evolution of ⁶-ox-LGE₂ was monitored by RP-HPLC and ¹H-NMR. In the ¹H-NMR spectrum, the disappearance of the vinyl hydrogen resonances corresponding to the *cis* C=C bond (H-5, 5.43 ppm; H-6, 5.51 ppm) and the appearance of vinyl hydrogen resonances corresponding to the *trans* double bond (H-6, H-7, 6.48~6.56 ppm), which appear as a second-order ABX₂ spin system ($\nu/J \approx 1$) coupled with the allylic hydrogens (H-5), support the structural conclusions. In the HPLC chromatogram, the peak for the major ox-LGE₂ diastereomer ($t_r = 49$ min) diminished gradually while another peak ($t_r = 53$ min) grew (Figure 5). The ¹H NMR spectrum of pure ⁶-ox-LGE₂ isolated by HPLC (Figure 6) confirmed the structure assigned.

Further confirmation of the ⁶-ox-LGE₂ structure assigned to the rearrangement product from the ox-LGE₂ diastereomers was provided by 2D NMR spectra (Figures S10-S13). Two-dimensional NMR experiments, including HMBC, COSY, HSQC and NOESY (Figures S10-S15), confirmed the allylically rearranged structure assigned to the diastereomeric products generated by decomposition of the ox-LGE₂ diastereomers. COSY (Figure S10) H-H and HSQC (Figure S11) C-H one-bond correlation supported the structure of ⁶-ox-LGE₂ deduced from ¹H NMR, and allowed accurate assignment of ¹H NMR and ¹³C NMR resonances even with the interference of a large solvent peak from CD₃CN. A HMBC experiment (Figure S12) supported similar connectivity to that of ox-LGE₂, i.e., strong correlation (Figure 3) between the C-9 carbonyl and the C-13 vinyl carbon of the lower side chain as well as C-8-H-13 and C-8-H-14 correlations indicating that the newly generated compound also has a methyl group adjacent to the carboxyl side chain. As expected, the atoms on the new *trans* double bond (atoms 6 and 7) showed strong correlation with nearby atoms as indicated in Figure S11. The strongest evidence supporting the proposed ⁶-ox-LGE₂ structure was provided by a NOESY experiment. In the NOESY (Figure S13), the H-10 methyl hydrogens only correlate (Figure 3) with the closest proton, which is H-7. Confirmation for the assignment of the resonances at 6.48~6.56 ppm to H-7 was indicated by the fact that the strongest peaks associated with H-10 are only found at 1.66 ppm and 6.54 ppm in contrast with the NOESY ox-LGD₂ (Figure S15) that only shows strong correlation (Figure 3) between H-10' and H-13'.

The facility with which the ox-LGE₂ diastereomers rearrange, in contrast with the stability of ox-LGD₂, is presumably associated with the acidity of the C-7 methylene hydrogens. Proton abstraction from C-7 by fluoride followed by protonation at C-5 provides a base catalyzed pathway for the isomerization of ox-LGE₂ to the more thermodynamically stable conjugated isomer ⁶-ox-LGE₂ that accompanies desilylation of **2p** with Bu₄NF (Scheme 7).

Partial separation of ox-LGD₂, two ox-LGE₂ diastereomers and the product of ox-LGE₂ rearrangement, ⁶-ox-LGE₂ (**2d**), could be accomplished by reversed-phase HPLC. Using CH₃CN: H₂O: acetic acid 35:65:0.1 (v/v) as eluent, ox-LGD₂ and the minor diastereomer of ox-LGE₂ (**2b**) eluted as a single peak at 44 min, then the major diastereomer of ox-LGE₂ (**2a**) eluted at 49 min, and finally rearranged ox-LGE₂, ⁶-ox-LGE₂ (**2d**), eluted at 53 min (Figure S16). The ¹H NMR spectrum (Figure S17) of the first peak from Figure S16 showed that it contains ox-LGD₂ (**1**) and the minor diastereomer **2b** of ox-LGE₂. This mixture was further resolved by HPLC eluting with MeOH: H₂O: acetic acid = 50:50:0.1 (Figure S18)

into two symmetrical peaks, the first being the minor diastereomer **2b** of ox-LGE₂ and the second being the same ox-LGD₂ diastereomer obtained from the desilylation with Bu₄NF. Presumably, the absolute configuration at C-15 produced enzymatically is S. The configuration at C-11 remains unknown. Apparently one diastereomer of ox-LGD₂ is favored both in the total synthesis and in its production *in vivo*. Thus, the fluoride catalyzed selective destruction of ox-LGE₂ was a fortunate discovery that readily allowed the isolation of pure ox-LGD₂. It seems likely that oxidation of LGE₂ that is cogenerated with LGD₂ in the nonenzymatic rearrangement of PGH₂ would produce ox-LGE₂ and also that this metabolite would readily rearrange to ⁶-ox-LGE₂. The availability of a pure sample of ⁶-ox-LGE₂ from our total synthesis should enable the development of analytical protocols for its detection in biological samples. On the other hand, if the production of LGD₂ is enzyme-catalyzed *in vivo*, it may be selectively generated from PGH₂, and neither ox-LGE₂ nor ⁶-ox-LGE₂ will be found because an enzyme-mediated rearrangement may not coproduce LGE₂.

A Physiological Role for the In Vivo Oxidation of Levuglandins

Whereas amyloid (A)β₁₋₄₂ incubated for 24 h with LGE₂ is toxic to primary cultures of cerebral neurons of mice, exposure of the neurons to Aβ₁₋₄₂ itself after 24 h incubation in the absence of LGE₂ has little or no effect.¹³ Brain levels of LG-protein lysyl adducts correlate with Alzheimer's disease (AD) severity.¹⁴ LG-lysyl adducts in proteins extracted from the hippocampus of brains from seven patients with clinical and pathological evidence of AD averaged 12.2-fold higher than in five age-matched controls. The levels of LG-lysyl adducts demonstrate a highly significant positive relationship ($r = 0.92$, $p < 0.0001$, $n = 12$) with the neurofibrillary tangle score according to the Braak and Braak method (Braak stage).³⁴ LG-lysyl adduct levels also are positively correlated ($r = 0.72$, $p < 0.01$, $n = 12$) with CERAD neuritic plaque score.³⁵

The oxidative conversion of LGs into ox-LGs *in vivo* provides a detoxification mechanism because ox-LGs lack the reactive γ-ketoaldehyde functional array of that accounts for their rapid formation of covalent adducts with biomolecules. Oxidative catabolism of LGs converts them into less reactive ox-LG end products. To be effective in protecting against the pathological consequences of LG-protein adduction, oxidative catabolism must be highly efficient. Even low levels of LGs promote the formation of the type of amyloid Aβ₁₋₄₂ oligomers that have been associated with neurotoxicity and are a pathologic hallmark of AD. Thus, oligomerization of Aβ by LGE₂ occurs with ratios of LGE₂ : Aβ of only 1 : 10.¹³ This suggests that intermolecular crosslinking cannot be the sole mechanism for oligomerization, and raises the possibility that the formation of LG adducts of Aβ serves as a seed to accelerate oligomerization. AD pathology is associated with a deficiency in the ability to detoxify endogenous aldehydes. Mitochondrial aldehyde dehydrogenase 2 (ALDH2) metabolizes aldehydes. In the Japanese population, ALDH2 deficiency is caused by a mutant allele of the *ALDH2* gene (*ALDH2*2*). In a large clinical study, the odds ratio for late onset AD in carriers of the *ALDH2*2* allele was almost twice that in noncarriers.³⁶ The discovery, now confirmed by total synthesis, that interception of LGs by oxidative catabolism to ox-LGs can compete with their adduction to proteins suggests that individuals with ALDH2

deficiency will exhibit elevated levels of LG-protein adducts compared to noncarriers of the *ALDH2*2* allele.

SUMMARY

Total synthesis played a pivotal role in the identification of levuglandins as products of the spontaneous rapid rearrangement of the prostaglandin endoperoxide PGH_2 and in our discovery of a free radical oxidative pathway that also produces levuglandins as well as a large family of non-prostanoid structural isomers referred to collectively as isolevuglandins (isoLGs).^{2,37–40} Pure samples of LGs and isoLGs available through total syntheses also enabled studies of their biological chemistry that had been presumed to be dominated by their rapid covalent adduction with primary amino groups in biomolecules. In view of the exceptionally high proclivity for LGs to form covalent adducts with biomolecules, a competing alternative fate, i.e., oxidation to ox-LGs *in vivo*, was unexpected. The recent discovery, now confirmed by unambiguous total synthesis, that oxidative metabolism of LGD_2 to ox- LGD_2 (**1**) competes *in vivo* with this covalent adduction chemistry provides presumptive evidence for the proposition that pathology associated with LGs and isoLGs, e.g., AD, may not solely be a consequence of their generation and adduction with biomolecules, but also deficiencies in their detoxification by oxidative metabolism, e.g., by ALDH, that converts them into less reactive ox-LG end products. ALDH deficiency correlates with an almost 2-fold increase in odds ratio for late onset AD. Although the cogeneration of ox- LGE_2 with ox- LGD_2 *in vivo* is expected, our observation that ox- LGE_2 readily undergoes allylic C=C bond migration, suggests that isolation of this metabolite of LGE_2 from biological samples may be elusive. Detection and isolation of the more thermodynamically stable rearrangement product, ⁶-ox- LGE_2 (**2d**), is now anticipated and will be facilitated by the availability of an authentic sample through the total synthesis reported herein.

EXPERIMENTAL SECTION

General Experimental Procedures

Nuclear magnetic resonance (NMR) spectra were acquired on either a Varian Inova AS400 operating at 400 and 100 MHz, 500 MHz Bruker Ascend Avance III HDTM equipped with ProdigyTM ultra-high sensitivity Multinuclear Broadband CryoProbe operating at 500 and 125 MHz for ^1H and ^{13}C , respectively, and are referenced internally according to residual solvent signals. All ESI mass spectra were obtained from Thermo Finnigan LCQ Deca XP and all high-resolution mass spectra were recorded on a Kratos AEI MS25 RFA high-resolution mass spectrometer at 20 eV. High performance liquid chromatography (HPLC) was performed on Shimadzu UFLC system equipped with a 5 μm Phenomenex Luna C-18 column. Flash column chromatography was performed on 230-400 mesh silica gel supplied by E. Merck with ACS grade solvent. Rf values are quoted for plates of thickness 0.25 mm. The plates were visualized with iodine, UV and phosphomolybdic acid reagents. All reactions were carried out under an argon atmosphere. All reagents were obtained commercially unless otherwise noted. Reactions were performed using glassware that was oven-dried at 120 °C. Air- and moisture-sensitive liquids and solutions were transferred via

syringe or stainless steel cannula. Except for tetrahydrofuran (THF), toluene and CH_2Cl_2 were distilled under a nitrogen atmosphere prior to use, all other solvent were purchased from commercial suppliers and used as received. (E)-tert-butyldimethyl((1-(tributylstannyl)oct-1-en-3-yl)oxy)silane (**5**) was prepared as described previously.⁴¹

tert-Butyl(4-iodobutoxy)dimethylsilane (**8**)

To a solution of tert-butyldimethylsilyl chloride (12.5 g, 0.083 mol) in CH_3CN (40 mL) was added NaI (18.7 g, 0.125 mol) and dry THF (50 mL). The mixture was stirred overnight at room temperature (rt) in the dark. After the reaction has completed, the solution was then filtered through a sintered-glass funnel and the solid was washed with 25% EtOAc in hexanes. The filtered clear wine red solution was then rinsed with 50% saturated $\text{Na}_2\text{S}_2\text{O}_3$ (3×15 mL) in a separatory funnel, the aqueous and organic layers were separated and sequentially extracted with 20% EtOAc in hexanes (2×50 mL). The combined organic layer was washed with brine, dried with Na_2SO_4 . Solvent was then removed by rotary evaporation and the crude product was purified by chromatography on a silica gel column eluting with 100% of hexanes ($R_f = 0.25$) to give **8** (24.3 g, yield = 93%) as a clear oil. **8**: $R_f = 0.25$ (hexanes); ^1H NMR (400 MHz, CDCl_3): δ 3.60 (t, 2H), 3.21 (t, 2H), 1.82 (m, 2H), 1.60 (m, 2H), 0.82 (s, 9H), 0.02 (s, 6H); ^{13}C NMR: Spectra corresponding to literature; HREIMS m/z 313.0487 $[\text{M}]^+$ (calcd for $\text{C}_{10}\text{H}_{22}\text{IOSi}$, 313.0485).

(Z)-7-((tert-Butyldimethylsilyl)oxy)hept-2-yne-1-ol (**9**)

Propargyl alcohol (1.79 g, 31.9 mmol), HMPA (22.8 g, 127 mmol, 4 equiv.) in 40 mL of dry THF was cooled to -40°C in an CH_3CN /dry ice bath. Butyl lithium (2.5 M, 26.0 mL, 2.0 equiv.) was added dropwise and the mixture was stirred at -40°C for about 1 h. **8** (10.0 g, 31.9 mmol) in THF (10 mL) at -40°C was added via cannula to the mixture. The mixture was slowly warmed to rt. After 4 h, the mixture was quenched with saturated aqueous NH_4Cl solution (20 mL), extracted with diethyl ether, washed with H_2O , then brine, then dried over anhydrous Na_2SO_4 and concentrated by rotary evaporation to afford clear yellow oil. This crude product was purified by flash chromatography using 10% EtOAc in hexanes ($R_f = 0.20$) to deliver a clear light yellow oil **9** 5.2 g (yield = 67.3%) as a light yellow oil. **9**: $R_f = 0.20$ (9:1 hexanes/EtOAc); ^1H NMR (500 MHz, CDCl_3): δ 4.24 (d, 2H), 3.62 (t, 2H), 2.24 (m, 2H), 1.55~1.59 (m, 4H), 0.89 (s, 9H), 0.05 (s, 6H); ^{13}C NMR (125 MHz, CDCl_3): δ 86.57, 78.61, 62.78, 51.58, 32.06, 26.10, 25.17, 18.70, 18.49, -5.15 ; ESIMS m/z 243.07 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{13}\text{H}_{27}\text{O}_2\text{Si}$, 243.17).

(Z)-7-((tert-Butyldimethylsilyl)oxy)hept-2-en-1-ol (**10**)

Nickel acetate tetrahydrate (7.14 g, 29 mmol) in 95% of EtOH (200 mL) was placed under a balloon of H_2 . Sodium borohydride (1.09 g, 29 mmol) in EtOH (8 mL) was added at rt. After 20 min, ethylenediamine (7.0 g, 116 mmol) was added, followed by adding alkyne **9** (7.0 g, 29 mmol) in EtOH (10.0 mL). The reaction was monitored by TLC. After about 3 h, the reaction was filter through a pad of silica gel. The filtrate EtOH solution was concentrated by rotary evaporation. The residue was extracted with diethyl ether, washed with H_2O , then brine, then dried over anhydrous Na_2SO_4 and concentrated by rotary evaporation to afford a clear oil. This crude product was purified by flash chromatography

(hexanes : EtOAc = 4 : 1, R_f = 0.25) gave alkene **10** (6.14 g, 87%) as a clear oil. **10**: R_f = 0.25 (4:1 hexanes/EtOAc); ^1H NMR (500 MHz, CDCl_3): δ 5.59 (m, 1H), 5.34 (m, 1H), 4.18 (d, 2H), 3.60 (t, 2H), 2.10 (m, 2H), 1.52 (m, 2H), 1.40 (m, 2H), 0.89 (s, 9H), 0.04 (s, 6H); ^{13}C NMR (125 MHz, CDCl_3): δ 132.94, 128.59, 63.00, 58.58, 32.32, 27.18, 25.98, 25.88, 18.37, -5.27; HREIMS m/z 243.1779 $[\text{M} - \text{H}]^+$ (calcd for $\text{C}_{13}\text{H}_{27}\text{O}_2\text{Si}$, 243.1780).

(*Z*)-7-((*tert*-Butyldimethylsilyl)oxy)hept-2-en-1-yl acetate (**11**)

A solution of alcohol **10** (5.8 g, 23.7 mmol) in pyridine (25.5 mL) was cooled to 0 °C and then acetic anhydride (7.0 mL, 3 equiv.) was slowly added. The mixture was stirred for 40 min at that temperature under argon, and then allowed to warm to rt. After 2 h the mixture (clear light yellow) was quenched by addition of EtOH (10 mL) and concentrated by rotary-evaporation to afford clear yellow oil. The residue was washed with 1 M HCl and concentrated to give **11** (6.46 g, 95%) as a clear oil: R_f = 0.20 (10:1 hexanes/Et₂O); ^1H NMR (400 MHz, CDCl_3): δ 5.63 (dt, 1H), 5.55 (dt, 1H), 4.61 (d, 2H), 3.60 (t, 2H), 2.12 (m, 2H), 2.06 (s, 3H), 1.51 (m, 2H), 1.43 (m, 2H), 0.89 (s, 9H), 0.04 (s, 6H); ^{13}C NMR (125 MHz, CDCl_3): δ 171.16, 135.43, 123.57, 63.09, 60.53, 32.48, 27.44, 26.11, 25.86, 21.16, 18.50, -5.13; HREIMS m/z 227.1832 $[\text{M} - \text{C}_2\text{H}_3\text{O}_2]^+$ (calcd for $\text{C}_{13}\text{H}_{27}\text{OSi}$, 227.1831). This product was used without further purification for the preparation of the hydroxy acid **12**.

(*Z*)-7-Hydroxyhept-5-enoic acid (**12**)

To a solution of **11** (7.86 g, 27.0 mmol) in acetone (33 mL) at 0 °C was added Jones reagent dropwise until the orange color persisted for 20 min. The reaction was then quenched by addition of isopropyl alcohol (20 mL), and then filtered through Celite to afford 7-acetoxyhept-5-ynoic acid after removal of solvents by rotary evaporation. The crude (*Z*)-7-acetoxyhept-5-enoic acid was dissolved in EtOAc (36 mL) and extracted into 10% NaOH (5 × 25 mL). The aqueous layers were collected and acidified with 6 M HCl to pH = 1, followed by extraction with EtOAc (5 × 50 mL). The organic layers were combined and washed with H₂O, then brine, then dried over anhydrous magnesium sulfate, filtered and concentrated by rotary evaporation to afford a clear oil **12** (2.63 g) that exhibited a single spot R_f = 0.3 by TLC with EtOAc. The yield is 68 % for the three steps. **12**: R_f = 0.30 (EtOAc); ^1H NMR (400 MHz, CDCl_3): δ 5.63 (dt, 1H), 5.55 (dt, 1H), 4.20 (d, 2H), 2.32 (t, 2H), 2.15 (t, 2H), 1.68 (m, 2H); HREIMS m/z 126.0681 $[\text{M} - \text{H}_2\text{O}]^+$ (calcd for $\text{C}_7\text{H}_{10}\text{O}_2$, 126.0681).

Di-*tert*-butyl(methyl)silyl (*Z*)-7-hydroxyhept-5-enoate (**13**)

Acid **12** (1.00 g, 6.94 mmol) in anhydrous THF (10 mL) under argon was treated with dry triethylamine (4.0 eq, 2.81 g, 27.3 mmol) at rt. And then di-*tert*-butylmethyl trifluoromethanesulfonate (2.23 g, 7.28 mmol) was added dropwise. After about 30 min, the solution was concentrated in vacuo and the residue was purified by flash chromatography with EtOAc/hexanes (1:4, R_f = 0.3) to give **13** (1.38 g, yield 67 %) as a clear oil. **13**: R_f = 0.30 (4:1 hexanes/EtOAc); ^1H NMR (400 MHz, CDCl_3): δ 5.60 (m, 1H), 5.44 (m, 1H), 4.10 (d, 2H), 2.28 (t, 2H), 2.08 (q, 2H), 1.66 (m, 2H), 0.95 (s, 18H), 0.25 (s, 2H); ^{13}C NMR (125

MHz, CDCl₃): δ 173.31, 131.62, 129.65, 58.33, 35.49, 27.51, 26.58, 24.84, 20.28, -7.51; HREIMS m/z 283.2089 [M - OH]⁺ (calcd for C₁₆H₃₁ O₂Si, 283.2093).

Di-tert-butyl(methyl)silyl (Z)-7-bromohept-5-enoate (7)

Methanesulfonyl chloride (343 mg, 3.0 mmol) was added dropwise to a chilled solution of **13** (500 mg, 0.166 mmol), triethylamine (337 mg, 3.33 mmol) in CH₂Cl₂ (6.0 mL) at -50 °C. The resulting white suspension was stirred for 45 min and then treated with a solution of lithium bromide (577 mg, 6.64 mmol) in THF (2.0 mL). The colorless mixture was then warmed slowly to -20 °C and stirred for 1 h. Then the reaction mixture was poured over H₂O and extracted with pentane. The pentane extracts were washed with H₂O, then brine, then dried and concentrated by rotary evaporation to deliver crude allylic bromide. The crude product was purified by flash chromatography with EtOAc/hexanes (1 : 25, R_f = 0.2) to give pure **7** (503 mg, 83.3 %) as a clear oil. **7**: R_f 0.20 (25:1 hexanes/EtOAc); ¹H NMR (400 MHz, CDCl₃): δ 5.76 (m, 1H), 5.59 (dt, 1H), 3.99 (d, 2H), 2.37 (t, 2H), 2.20 (q, 2H), 1.74 (m, 2H), 1.02 (s, 18H), 0.32 (s, 3H); ¹³C NMR (100 MHz, CDCl₃): δ 173.08, 134.80, 126.47, 35.72, 27.73, 27.15, 26.49, 24.71, 20.49, -7.28; HREIMS m/z 305.0575, 307.0553 [M - C₄H₉]⁺ (calcd for C₁₂H₂₂BrO₂Si, 305.0572, 307.0552).

Three-component coupling product 4 and its regioisomer 4'

A solution of (E)-tert-butyldimethyl((1-(tributylstannyl)oct-1-en-3-yl)oxy)silane (**5**)⁴¹ (160 mg, 0.3 mmol) in dry THF (1.0 mL) at -78 °C was treated with n-BuLi (1.6 M, 0.12 mL, 0.3 mmol). After 40 min, the light yellow solution was transferred dropwise via a cannula to a solution of copper cyanide (14 mg, 0.15 mmol) in anhydrous THF (2.0 mL) at -78 °C. The resulting clear solution was stirred at -78 °C for 2 h. Then a solution of di-tert-butyl acetylenedicarboxylate (**6**) (68 mg, 0.3 mmol) in anhydrous THF (1.0 mL) was slowly added via cannula to the cuprate at -78 °C. The mixture was stirred at -78 °C for 50 min. Freshly distilled HMPA (480 mg, 2.7 mmol) in THF was then added. After 15 min, Pd(PPh₃)₄ (12 mg, 0.01 mmol) in THF (1.0 mL) was added by cannula, followed by the addition of **6** (55 mg, 0.15 mmol) in THF (1.0 mL). Stirring was continued overnight at -78 °C and the mixture was then warmed to -45 °C and stirred for one more hour. Then the reaction mixture was quenched by addition of aqueous saturated NH₄Cl/NH₄OH (pH 9, 5 mL), and slowly warmed to 0 °C. Diethyl ether was used to extract the mixture. The crude product was purified by flash chromatography with hexanes/Et₂O (40 : 1 to 20 : 1) to give **4** and **4'** (60 mg, yield = 54%) as clear oil. R_f = 0.25 (20:1 hexanes/Et₂O). Product **4** and **4'** were then separated by HPLC (Luna C18 5 μ m, 10 $\times$ 250 mm, gradient elution with CH₃CN/2-propanol).

(4Z,7Z,9E)-7,8-Di-tert-butyl 1-(di-tert-butyl(methyl)silyl) 11-((tert-butyldimethylsilyl)oxy)hexadeca-4,7,9-triene-1,7,8-tricarboxylate (4)

¹H NMR (500 MHz, CDCl₃): δ 6.41 (d, 1H, J = 15.8 Hz), 5.99 (dd, 1H, J = 15.8 Hz, J = 5.2 Hz), 5.40 (m, 1H), 5.31 (m, 1H), 4.22 (q, 1H, J = 5.2 Hz), 3.11 (d, 2H), 2.35 (t, 2H), 2.15 (m, 2H), 1.72 (m, 2H), 1.49 (m, 20H), 1.31~1.26 (m, 6H), 1.01 (s, 18H), 0.89 (m, 12H), 0.31 (s, 3H), 0.03 (d, 6H); ¹³C NMR (125 MHz, CDCl₃): δ 173.18, 167.65, 166.41, 141.92, 139.75, 130.56, 130.27, 126.92, 122.01, 81.77, 81.29, 73.08, 38.15, 35.84, 31.95, 28.22,

28.15, 27.66, 27.05, 26.54, 25.99, 25.13, 24.81, 22.74, 20.41, 18.34, 14.17, -4.23, -4.66, -7.36; HREIMS m/z 694.4463 $[M - C_4H_8]^+$ (calcd for $C_{38}H_{70}O_7Si_2$, 694.4460).

(5Z,7E)-5,6-Di-tert-butyl 1-(di-tert-butyl(methyl)silyl) 9-((tert-butyldimethylsilyl)oxy)-4-vinyltetradeca-5,7-diene-1,5,6-tricarboxylate (4')

1H NMR (500 MHz, $CDCl_3$): δ 6.39 (d, 1H, $J = 15.8$ Hz), 5.95 (d, 1H, $J = 17.0$ Hz), 5.89 (1H, ddd, $J = 9.9, 6.2, 2.6$ Hz), 5.04 (d, 2H, $J = 11.8$ Hz), 4.21 (s, 1H), 3.27 (q, 1H, $J = 7.5$ Hz), 2.46~2.17 (m, 2H), 1.77~1.58 (m, 2H), 1.53 (m, 2H), 1.50 (s, 9H), 1.48(s, 9H), 1.37~1.18 (m, 6H), 1.01 (s, 18H) 0.90 (s, 9H), 0.87(t, 3H), 0.31 (s, 3H), 0.04 (d, $J = 12.4$ Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 173.00, 167.40, 166.96, 141.25, 138.60, 137.09, 136.07, 121.16, 116.19, 81.77, 81.71, 73.07, 43.68, 38.19, 36.22, 32.68, 31.98, 28.25, 28.15, 27.66, 26.02, 24.84, 24.78, 23.49, 22.76, 20.39, 18.35, 14.18, -4.20, -4.66, -7.37; ESIMS m/z 773.20 $[M + Na]^+$ (calcd for $C_{42}H_{78}O_7Si_2Na$, 773.52).

(E)-di-tert-butyl(methyl)silyl 5-(4-(3-((tert-butyldimethylsilyl)oxy)oct-1-en-1-yl)-2,5-dioxo-2,5-dihydrofuran-3-yl)hept-6-enoate (3')

To a solution of 4' (42 mg, 0.056 mmol) in toluene (5.0 mL) was added SiO_2 (360 mg). The solution was boiled under reflux under argon for about 6 h, then cooled to rt, then diluted with 10 mL CH_2Cl_2 , and filtered through a pad of Celite. The solution was concentrated under vacuum. The crude product 3 was used for the next step without further purification (33.0 mg, yield = 95%). 3': Clear Oil; R_f 0.80 (10:1 hexanes/EtOAc); 1H NMR (500 MHz, $CDCl_3$): δ 7.28 (ddd, 1H, $J = 4$ Hz, $J = 5$ Hz), 6.57 (ddd, 1H, $J = 16$ Hz, $J = 7$ Hz, $J = 2$ Hz), 5.96 (m, 1H), 5.13 (m, 2H), 4.37 (t, 1H), 3.43 (q, 1H), 2.34 (m, 2H), 1.82 (m, 2H), 1.66 (m, 1H) 1.55 (m, 3H), 1.37~1.25 (m, 6H), 1.00 (s, 18H), 0.93 (d, 9H), 0.89 (t, 3H) 0.31 (d, 3H), 0.09 (s, 3H), 0.04(s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 172.49, 164.75, 164.26, 149.94, 140.31, 136.53, 135.86, 117.85, 114.31, 72.28, 41.18, 37.49, 35.63, 31.95, 31.82, 27.49, 25.85, 24.64, 23.25, 22.55, 20.25, 18.25, 14.02, -4.64, -4.76, -7.52; ESIMS m/z 643.20 $[M + Na]^+$ (calcd for $C_{34}H_{60}O_6Si_2Na$, 643.38).

(Z)-Di-tert-butyl(methyl)silyl 7-(4-((E)-3-((tert-butyldimethylsilyl)oxy)oct-1-en-1-yl)-2,5-dioxo-2,5-dihydrofuran-3-yl)hept-5-enoate (3)

To a solution of 4 (21 mg, 0.028 mmol) in toluene (2.5 mL) was added SiO_2 (200 mg). The solution was boiled under reflux under argon for about 6 h, then cooled to rt, then diluted with 5 mL CH_2Cl_2 , and filtered through a pad of Celite. The solution was concentrated under vacuum. The crude product 3 was used for the next step without further purification (16.0 mg, yield= 91%). 3: Clear Oil; R_f 0.80(10:1 hexanes/EtOAc); 1H NMR (500 MHz, $CDCl_3$): δ 7.22 (dd, 1H, $J = 16$ Hz, $J = 4.0$ Hz), 6.54 (dt, 1H, $J = 16$ Hz), 5.56 (dt, 1H), 5.38 (dt, 1H), 4.37 (dd, 1H, $J = 4.0$ Hz), 3.26 (d, 2H), 2.36 (t, 2H), 2.21 (m, 2H), 1.72 (tt, 2H) 1.56 (m, 2H), 1.37~1.25 (m, 6H), 1.02 (s, 18H), 0.93 (s, 9H), 0.88 (t, 3H) 0.32 (s, 3H), 0.06 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 173.05, 165.81, 164.67, 149.75, 138.12, 136.68, 133.33, 122.62, 114.64, 72.48, 37.66, 35.69, 31.96, 27.65, 26.92, 25.98, 24.79, 24.71, 22.70, 22.48, 20.41, 18.38, 14.18, -4.45, -4.64, -7.36; HREIMS m/z 563.3226 $[M - C_4H_9]^+$ (calcd for $C_{30}H_{51}O_6Si_2$, 563.3224).

(E)-Di-tert-butyl(methyl)silyl 5-(4-(3-((tert-butyldimethylsilyl)oxy)oct-1-en-1-yl)-5-hydroxy-5-methyl-2-oxo-2,5-dihydrofuran-3-yl)hept-6-enoate (1p') and (E)-di-tert-butyl(methyl)silyl 5-(4-(3-((tert-butyldimethylsilyl)oxy)oct-1-en-1-yl)-2-hydroxy-2-methyl-5-oxo-2,5-dihydrofuran-3-yl)hept-6-enoate (2p')

Methyl lithium (57.5 μ L, 1.6 M in diethyl ether, 3.0 equiv.) was added to **3'** (19 mg) in dry THF (3.5 mL) at -94°C . The reaction was kept at -94°C for 15 min. The reaction mixture was quenched with aqueous NH_4Cl . Diethyl ether was used to extract the mixture. The crude product was purified by flash chromatography with hexanes/EtOAc (5 : 1, R_f = 0.3) to give a mixture of hydroxylactones **1p'** and **2p'** (8.2 mg, yield = 52% based on recovered starting material) as a clear oil. ^1H NMR data showed there are sets of two peaks, which indicates that the product is a mixture of two hydroxylactones (1:1) **1p'** and **2p'**: R_f = 0.30 (5:1 hexanes/EtOAc); ^1H NMR (500 MHz, CDCl_3): δ 7.02 (m, 0.5H, isomer 1), 6.54 (m, 1H, isomer 2), 6.34 (dd, 0.5H, isomer 1), 6.06 (m, 1H, isomer 1), 5.91 (dddt, 1H, isomer 2), 5.08 (m, 2H), 4.31 (m, 1H), 3.26 (m, 1H), 2.33 (m, 2H), 1.79 (q, 2H), 1.63~1.72 (m, 3H), 1.49~1.62 (m, 4H), 1.23~1.39 (m, 6H), 1.00 (m, 18H), 0.93 (m, 9H), 0.88 (m, 3H) 0.30 (m, 3H), 0.05 (m, 6H); ESIMS m/z 659.27 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{35}\text{H}_{64}\text{O}_6\text{Si}_2\text{Na}$, 659.41).

(Z)-Di-tert-butyl(methyl)silyl 7-(4-((E)-3-((tert-butyldimethylsilyl)oxy)oct-1-en-1-yl)-5-hydroxy-5-methyl-2-oxo-2,5-dihydrofuran-3-yl)hept-5-enoate (1p) and (Z)-di-tert-butyl(methyl)silyl 7-(4-((E)-3-((tert-butyldimethylsilyl)oxy)oct-1-en-1-yl)-2-hydroxy-2-methyl-5-oxo-2,5-dihydrofuran-3-yl)hept-5-enoate (2p)

Methyl lithium (18 μ L, 1.6 M in diethyl ether, 1.2 equiv.) was added to **3** (15 mg) in dry THF (5 mL) at -94°C . The reaction was kept at -94°C for 15 min. The reaction mixture was quenched with aqueous NH_4Cl . Diethyl ether was used to extract the mixture. The crude product was purified by flash chromatography with hexanes/EtOAc (5 : 1, R_f = 0.3) to give a 1:6 mixture of hydroxylactones **1p** and **2p** based on the integrated ^1H NMR peak areas for resonances centered at δ 6.50 and 6.32 (Figure S2), corresponding to H-13 in **1p** and **2p** respectively (4 mg, yield = 57% based on unrecovered starting material) as a clear oil. ^1H NMR data showed there are sets of two peaks, which indicates that the product is a mixture of two hydroxy lactones. Major regioisomer **2p**: R_f = 0.30 (5:1 hexanes/EtOAc); ^1H NMR (500 MHz, CDCl_3): δ 6.96 (dt, 1H), 6.30 (d, 1H), 5.53 (m, 1H), 5.44 (m, 1H), 4.27 (m, 1H), 3.21 (m, 2H), 2.36 (t, 2H), 2.20 (m, 2H), 1.72 (m, 2H), 1.65 (s, 3H), 1.51 (m, 2H), 1.34~1.23 (m, 6H), 1.02 (s, 18H), 0.92 (s, 9H), 0.93~0.86 (m, 3H) 0.32 (s, 3H), 0.05 (d, 6H); ^{13}C NMR (125 MHz, CDCl_3): δ 173.56, 169.38, 158.13, 141.89, 132.18, 124.45, 123.98, 115.10, 104.51, 72.81, 38.10, 35.67, 32.02, 29.85, 27.64, 26.04, 24.81, 24.71, 24.34, 24.00, 22.74, 20.41, 18.41, 14.20, -4.31 , -4.64 , -7.35 ; 2D-NMR, COSY, NOESY, HMBC, HSQC (Figures S3-S6); HREIMS m/z 636.4103 $[\text{M}]^+$ (calcd for $\text{C}_{35}\text{H}_{64}\text{O}_6\text{Si}_2$, 636.4241); m/z 579.3536 $[\text{M} - \text{C}_4\text{H}_9]^+$ (calcd for $\text{C}_{31}\text{H}_{54}\text{O}_6\text{Si}_2$, 579.3539); m/z 618.4133 $[\text{M} - \text{H}_2\text{O}]^+$ (calcd for $\text{C}_{35}\text{H}_{62}\text{O}_5\text{Si}_2$, 618.4136).

(E)-5-(5-Hydroxy-4-(3-hydroxyoct-1-en-1-yl)-5-methyl-2-oxo-2,5-dihydrofuran-3-yl)hept-6-enoic acid (1') and (E)-5-(2-hydroxy-4-(3-hydroxyoct-1-en-1-yl)-2-methyl-5-oxo-2,5-dihydrofuran-3-yl)hept-6-enoic acid (2')

Method A—A solution of **1p'** and **2p'** (9 mg, 0.014 mmol) in THF (300 μ L) was cooled to 0 °C and then tetra-n-butylammonium fluoride (Bu_4NF , 1.0 M in THF with 5% H_2O , 148 μ L) was added. After stirring at 0 °C for 15 min, the cold bath was removed and the clear tan solution was stirred at rt overnight. The progress of the reaction was monitored by TLC developing with 60% EtOAc in hexanes containing 1% acetic acid. The reaction mixture was distributed between saturated aqueous sodium bicarbonate (9 mL) and n-pentane (9 mL). The organic layer was extracted with saturated aqueous sodium bicarbonate, and then discarded. The combine aqueous extracts were carefully acidified to pH 3 by dropwise addition of 1.0 M HCl, and then extracted with diethyl ether (5×9 mL). The organic extracts were combined, dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by flash chromatography with 60% EtOAc in hexanes containing 1% acetic acid ($R_f = 0.3$) to give mixture **1'** and **2'**. (4.2 mg, yield = 81%) as a clear oil.

Method B—A mixture of **1p'** and **2p'** (3.0 mg, 0.0047 mmol) was treated with concentrated aqueous hydrofluoric acid (49% w/v) in CH_3CN (10% v/v, 525 μ L) in a polyethylene vial. The progress of the reaction was monitored by TLC developing with 60% EtOAc in hexanes containing 1% acetic acid. After 1.5 h, when the deprotection was complete by TLC, the solution was poured H_2O (6 mL) and extracted with CH_2Cl_2 (6 mL) in a polyethylene container. The organic layer was separated and extracted with brine (6 mL). The aqueous layers were then back extracted with CH_2Cl_2 (3×6 mL). The organic extracts were combined, dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by flash chromatography with 60% EtOAc in hexanes containing 1% acetic acid ($R_f = 0.3$) to give a mixture of **1'** and **2'** (1.7 mg, yield = 99.6%) as a clear oil. Two regioisomers **1'** and **2'**: $R_f = 0.30$ (4:6 hexanes/EtOAc with 1% AcOH); ^1H NMR (500 MHz, CDCl_3): δ 6.51 (d, 2H), 6.06 & 5.95 (m, 1H), 5.06 (m, 2H), 4.36 & 4.32 (m, 1H), 3.35 & 3.25 (m, 1H), 2.33 (m, 2H), 1.84 & 1.77 (m, 2H), 1.69~1.72 (m, 3H), 1.48~1.65 (m, 4H), 1.19~1.45(6H), 0.89 (m, 3H); ESIMS m/z 365.2 $[\text{M} - \text{H}]^-$ (calcd for $\text{C}_{20}\text{H}_{29}\text{O}_6$, 365.2).

(Z)-7-(5-Hydroxy-4-((E)-3-hydroxyoct-1-en-1-yl)-5-methyl-2-oxo-2,5-dihydrofuran-3-yl)hept-5-enoic acid (ox-LGD₂, 1), (Z)-7-(2-hydroxy-4-((E)-3-hydroxyoct-1-en-1-yl)-2-methyl-5-oxo-2,5-dihydrofuran-3-yl)hept-5-enoic acid (ox-LGE₂, 2) and (E)-7-(2-hydroxy-4-((E)-3-hydroxyoct-1-en-1-yl)-2-methyl-5-oxo-2,5-dihydrofuran-3-yl)hept-6-enoic acid (6-ox-LGE₂, 2d)

Method A—A mixture of **1p** and **2p** (9 mg, 0.0014 mmol) in THF (300 μ L) was cooled to 0 °C and then Bu_4NF (1.0 M in THF with 5% H_2O , 148 μ L) was added. After stirring at 0 °C for 15 min, the cold bath was removed and the clear tan solution was stirred at rt overnight. The progress of the reaction was monitored by TLC developing with 60% EtOAc in hexanes containing 1% acetic acid. The reaction mixture was distributed between saturated aqueous sodium bicarbonate (9 mL) and n-pentane (9 mL). The organic layer was extracted with saturated aqueous sodium bicarbonate, and then discarded. The combine aqueous extracts were carefully acidified to pH 3 by dropwise addition of 1.0 M HCl, and then extracted with diethyl ether (5×9 mL). The organic extracts were combined, dried over

Na₂SO₄ and concentrated in vacuo. The crude product was purified by flash chromatography with 60% EtOAc in hexanes containing 1% acetic acid (*R*_f = 0.3) to give ox-LGD₂ (**1**, 1.0 mg, yield = 18%) as a clear oil: ¹H NMR (500 MHz, CD₃CN, 1.90 ppm): δ 6.47 (d, 1H), 6.40 (dd, 1H), 5.40 (m, 1H), 5.39 (m, 1H), 4.18 (brt, 1H), 3.00 (brt, 2H), 2.25 (m, 2H) 2.14 (m, 2H), 1.60 (m, 2H), 1.58 (s, 3H), 1.48 (m, 2H), 1.36 (m, 1H), 1.22~1.33(5H), 0.85 (t, 3H); 2D-NMR: COSY, NOESY (Figures S14 and S15); MS (ESI): *m/z* calcd for C₂₀H₂₉O₆ (M-H), 365.2, found 365.2; HREIMS *m/z* 348.1936 [M - H₂O]⁺ (calcd for C₂₀H₂₈O₅, 348.1937).

Method B—A mixture of **1p** and **2p** (3.0 mg, 0.0047 mmol) was treated with concentrated aqueous hydrofluoric acid (49% w/v) in CH₃CN (10% v/v, 525 μL) in a polyethylene vial. The progress of the reaction was monitored by TLC developing with 60% EtOAc in hexanes containing 1% acetic acid. After 1.5 h, when the deprotection was complete by TLC, the solution was poured into H₂O (6 mL) and extracted with CH₂Cl₂ (6 mL) in a polyethylene container. The organic layer was separated and extracted with brine (6 mL). The aqueous layers were then back extracted with CH₂Cl₂ (3 × 6 mL). The organic extracts were combined, dried over Na₂SO₄ and concentrated in vacuo. The crude product was purified by flash chromatography with 60% EtOAc in hexanes containing 1% acetic acid (*R*_f = 0.3) to give a mixture of ox-LGD₂ and ox-LGE₂ (1.2 mg, yield = 70%) as a clear oil. TLC: *R*_f = 0.30 (4:6 hexanes/EtOAc with 1% AcOH); ox-LGE₂ (**2**, major diastereomer): ¹H NMR (500 MHz, CD₃CN, 1.94 ppm): δ 6.79 (dd, 1H), 6.32 (d, 1H), 5.51 (m, 1H), 5.43 (m, 1H), 4.14 (m, 1H), 3.21 (m, 2H), 2.29 (m, 2H) 2.18 (m, 2H), 1.66 (m, 2H), 1.56 (s, 3H), 1.48 (m, 2H), 1.30 (m, 1H), 1.25~1.35(5H), 0.88 (t, 3H); ¹³C NMR (125 MHz, CDCl₃): δ 174.51, 170.37, 160.65, 141.55, 132.34, 125.47, 124.14, 117.05, 105.72, 72.54, 37.97, 33.77, 32.44, 27.28, 25.86, 25.27, 24.48, 24.13, 23.29, 14.29; 2D-NMR: COSY, HMBC, HSQC (Figures S7-S9); ESIMS *m/z* 365.2 [M - H]⁻ (calcd for C₂₀H₂₉O₆, 365.2); ⁶-ox-LGE₂ (**2d**): ¹H NMR (500 MHz, CD₃CN, 1.94 ppm): δ 6.86 (dd, 1H), 6.51 (m, 2H), 6.43 (d, 1H), 4.14 (m, 1H), 2.29 (m, 4H), 1.64 (s, 3H), 1.59 (m, 2H), 1.41 (m, 1H), 1.51 (m, 2H), 1.26~1.36(5H), 0.88 (t, 3H); ¹³C NMR (125 MHz, CDCl₃): δ 174.78, 169.78, 154.50, 144.21, 141.61, 121.41, 119.53, 117.90, 116.71, 104.48, 100.57, 72.30, 37.65, 33.83, 33.73, 32.09, 28.32, 25.63, 25.50, 24.70, 22.93, 13.93; 2D-NMR : COSY, NOESY, HMBC, HSQC (Figures S12-S15); ESIMS *m/z* 365.2 [M - H]⁻ (calcd for C₂₀H₂₉O₆, 365.2); HREIMS *m/z* 348.1933 [M - H₂O]⁺ (calcd for C₂₀H₂₈O₅, 348.1937).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. HMBC (red) and NOESY (blue) correlations distinguishing the structures of **1p** and **2p**.

Figure 2.

Comparison of ¹H NMR spectra of the ox-LGD₂ chemical shift of CHD₂CN was set at 1.90 ppm (A) at 500 MHz from total synthesis and (B) at 600 MHz extracted from *Gracilaria edulis*,⁴ from Kanai, Y., Hiroki, S., Koshino, H., Konoki, K., Cho, Y., Cayme, M., Fukuyo, Y., and Yotsu-Yamashita, M. *J Lipid Res* **2011**52, 2245-2254. © the American Society for Biochemistry and Molecular Biology. (C) comparison of chemical shifts.

Figure 3.
HMBC (red) and NOSEY (blue) correlations distinguishing the structures of **1**, **2** and **2d**.

Figure 4.
 ^1H NMR spectrum of ox-LGD₂ and ox-LGE₂ mixture from HF-treated reaction.

Figure 5.
Evolution of ox-LGs in CD_3CN : (Left) HPLC chromatograph; (Right) ^1H NMR spectrum;
A: spectrum taken immediately after purification, B: after standing in CD_3CN for 24 h, C:
48 h, D: 7 days, E: 14 days.

Figure 6.
 ^1H NMR spectrum of $\Delta^6\text{-ox-LGE}_2$ (2d).

Scheme 1.
Generation and proposed structure of ox-LGD2

Scheme 2.
Synthetic design for ox-LGD₂

Scheme 3.
 Synthesis of the upper side chain precursor

Scheme 4. Concise total synthesis of ox-LGD₂

Scheme 5.
Model study of three-component coupling

Scheme 6.
 γ -Isomer model study.

Scheme 7.
Rearrangement of ox-LGE₂.

Table 1

Generation of 1 and 2 by desilylation of 1p and 2p.

Reagent	Products Total Isolated Yield (%)	Products (NMR Yield, %)	
		ox-LGD ₂	ox-LGE ₂
Bu ₄ NF	18	100	0
HF	70	14	86