Total Synthesis of Iso[4]-levuglandin E_2

Ganesamoorthy Subbanagounder,† Robert G. Salomon,*,† Krishna K. Murthi,†
Cynthia Brame,‡ and L. Jackson Roberts, II‡

Department of Chemistry, Case Western Reserve University, Cleveland, Ohio 44106-7078, and the Departments of Pharmacology and Medicine, Vanderbilt University, Nashville, Tennessee 37232-6602

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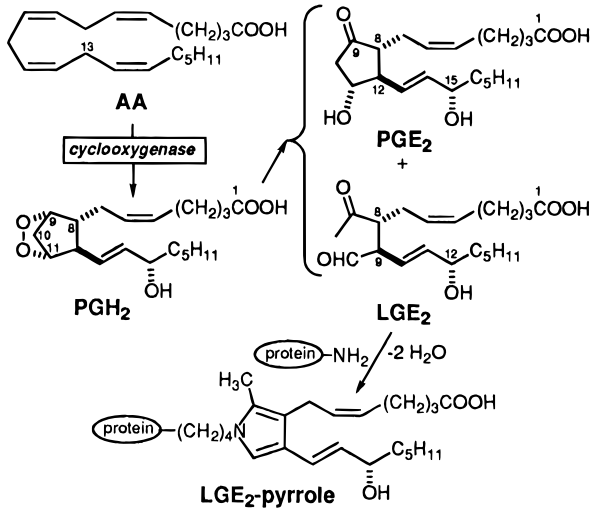
Iso[4]-levuglandin E_2 (iso[4]-LGE_2, 5-acetyl-6-formyl-9-hydroxy-7(*E*),11(*Z*)-heptadecadienoic acid) is a structural isomer of levuglandin E_2 (8-acetyl-9-formyl-12-hydroxy-5(*Z*),10(*E*)-heptadecadienoic acid) that we postulate to be generated during autoxidation of arachidonic acid or arachidonate esters. To facilitate detection and identification of iso[4]-LGE_2 in biological samples, a succinct total synthesis was developed that depends on conjugate addition of a higher order vinyl cyanocuprate to a γ-alkoxy enone to provide a key carbon−carbon bond-forming step. Bismethoxime pentafluorobenzyl ester trimethylsilyl ether derivatives of LGE_2 and iso[4]-LGE_2 were prepared and shown to be suitable for mass spectral identification of these highly oxidized lipids.

Introduction

Levuglandin (LG) E_2 is a highly oxidized lipid that is cogenerated[1] with prostaglandins (PGs),[2] e.g., PGE_2 (Scheme 1), by nonenzymatic rearrangements of the endoperoxide, PGH_2, that occur readily ($t_{1/2}$ = 5 min at 37 °C) under the conditions of its cyclooxygenase-promoted biosynthesis from arachidonic acid (AA). LGE_2 binds covalently with proteins[3] forming protein-bound pyrrole derivatives (LGE_2−pyrrole).[4] Using polyclonal antibodies against LGE_2−pyrrole, we recently detected LGE_2−protein adducts in human blood.[5] We also found that modification of low-density lipoproteins (LDLs) by adduction with LGE_2 results in receptor-mediated recognition, uptake, and degradation by macrophages. The recognition and processing of LGE_2−LDL by macrophages closely resemble that of oxidized LDL, and oxidized LDL competitively inhibits uptake of LGE_2−LDL.[6] This suggested the intriguing possibility that oxidative damage of LDLs involves generation of LGE_2 and its adduction with LDL protein. Since unregulated uptake of oxidized LDL is a prominent event in atherogenesis, it may be significant that higher levels of LGE_2−protein adducts were detected in the blood of atherosclerosis patients than in healthy controls.[5]

A nonenzymatic, free radical, oxidative pathway was uncovered recently that generates phospholipid endoperoxides, e.g., the 2-lysophosphatidylcholine (PC) ester 8-epi-PGH_2−PC, from arachidonyl phospholipids, e.g., AA−PC (Scheme 2).[7] In analogy with the conversion of

Scheme 1



PGH_2 into LGE_2, we expected that nonenzymatic rearrangement of 8-epi-PGH_2-PC would produce an LG−phospholipid, 8-epi-LGE_2−PC, and that Paal−Knorr condensation[8] with lysyl amino groups of LDL protein in conjunction with hydrolytic release of lysophosphatidylcholine would generate the same LGE_2−pyrrole (Scheme 2) as that produced by the cyclooxygenase pathway (Scheme 1). We recently verified that *in vitro* nonenzymatic free radical oxidation of LDL[9] generates posttranslational protein modifications that are immunoreactive toward antibodies raised against LGE_2−protein adducts.[10]

The Isolevuglandin Hypothesis. Since both the enzymatic and free radical pathways can generate LGE_2−pyrrole, detection of this protein modification *in vivo* cannot distinguish between the operation of these two oxidative pathways. The cyclooxygenase pathway only can produce levulinaldehydes with prostaglandin side chains (Scheme 1). However, because hydrogen atom

† Case Western Reserve University.
‡ Vanderbilt University.
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(1) Salomon, R. G.; Miller, D. B.; Zagorski, M. G.; Coughlin, D. J. *J. Am. Chem. Soc.* **1984**, *106*, 6049.
(2) (a) Hamberg, M.; Samuelsson, B. *Proc. Natl. Acad. Sci. U.S.A.* **1973**, *70*, 899. (b) Hamberg, M.; Svensson, J.; Wakabayashi, T.; Samuelsson, B. *Ibid.* **1974**, *71*, 345. (c) Nugteren, D. H.; Hazelhof, E. *Biochim. Biophys. Acta* **1973**, *326*, 488. (d) Raz, A.; Kenig-Wakshal, R.; Schwartzman, M. *Biochim. Biophys. Acta* **1977**, *488*, 322. (e) Nugteren, D. H.; Christ-Hazelhof, E. *Adv. Prostaglandin Thromboxane Res.* **1980**, *6*, 129.
(3) Salomon, R. G.; Jirousek, M. B.; Ghosh, S.; Sharma, R. B. *Prostaglandins* **1987**, *34*, 643.
(4) Iyer, R.; Kobierski, M. E.; Salomon, R. G. *J. Org. Chem.* **1994**, *59*, 6038.
(5) Salomon, R. G.; Subbanagounder, G.; O'Neil, J.; Kaur. K.; Smith, M. A.; Hoff, H. F.; Perry, G.; Monnier, V. M. *Chem. Res. Toxicol.* **1997**, *10*, 536.
(6) Hoppe, G.; Subbanagounder, G.; O'Neil, J.; Salomon, R. G.; Hoff, H. F. *Biochim. Biophys. Acta* **1997**, *1344*, 1.
(7) Morrow, J. D.; Harris, T. M.; Roberts II, L. J. *Anal. Biochem.* **1990**, *184*, 1.
(8) Jackson, A. H. in *Comprehensive Organic Chemistry*; Barton, D., Ollis, D. W., Eds.; Pergamon: New York, 1980; Vol. 4, p 276.
(9) Lynch, S. M.; Morrow, J. D.; Roberts, L. J., II; Freo, B. *J. Clin. Invest.* **1994**, *93*, 998.
(10) Salomon, R. G.; Subbanagounder, G.; Singh, U.; O'Neil, J.; Hoff, H. F.; *Chem. Res. Toxicol.* **1997**, *10*, 750−759.

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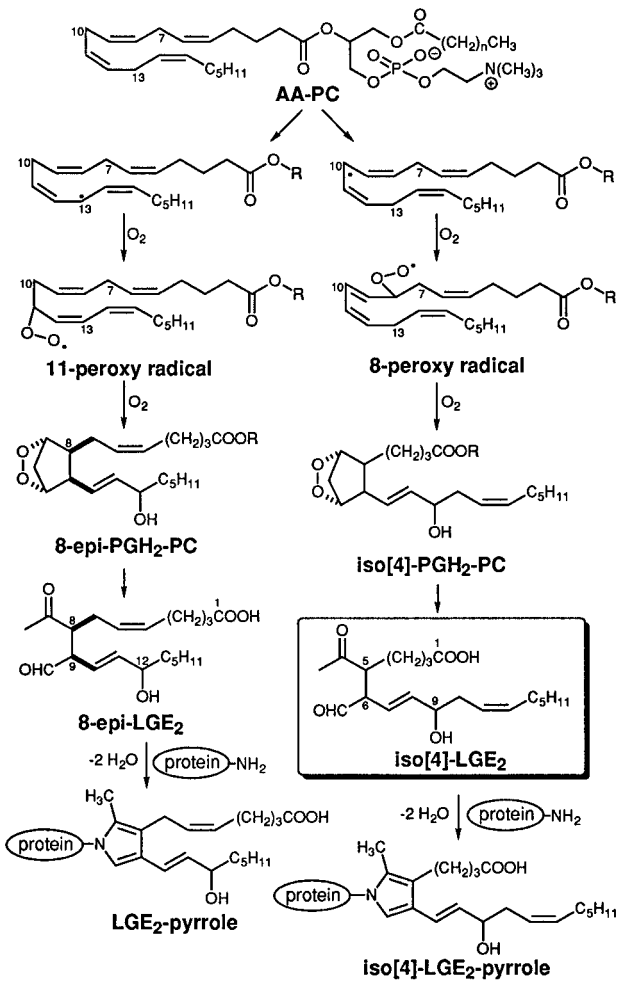
Scheme 2



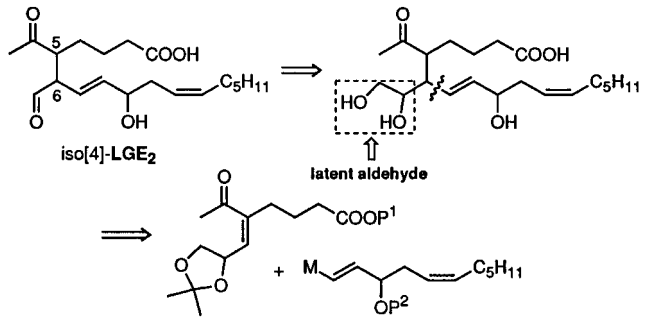
abstraction can occur nonregioselectively at any doubly allylic methylene, the free radical pathway can also generate levulinaldehyde derivatives with different side chains. For example, hydrogen atom abstraction from the 10-position of AA−PC followed by cyclization of an intermediate 8-peroxy radical could lead to iso[4]-LGE_2 (Scheme 2), where the number in brackets signifies the length of the carboxylic side chain appended to a levulinaldehyde core. Thus, in analogy with the conversion of PGH_2 into LGE_2, we expect that nonenzymatic rearrangement of iso[4]-PGH_2−PC[11] will produce iso[4]-LGE_2−PC, and this γ-keto aldehyde will form iso[4]-LGE_2−pyrrole by phospholipolysis and covalent adduction to proteins (Scheme 2).

In the present report, a concise total synthesis of iso[4]-LGE_2 is described that provides a practical source of adequate supplies for biological testing, authentic samples for mass spectral identification of derivatives produced from naturally occuring iso[4]-LGE_2 and its protein adducts, and synthetic protein adducts that we are using to develop an immunoassay in order to test the isolevuglandin hypothesis.

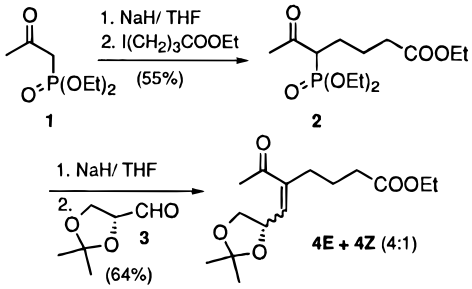
Results and Discussion

Synthetic Design. A convergent synthetic strategy (Scheme 3) similar to that used previously for a total

Scheme 3



Scheme 4



synthesis of LGE_2 was adopted.[12] Thus, a vicinal diol is exploited as a latent aldehyde and 1,4-addition of a vinyl nucleophile to a γ-alkoxy enone is used as the key carbon−carbon bond-forming step. Because two (C5 and C6) of the three stereocenters in iso[4]-LGE_2 will be destroyed upon condensation with ϵ-amino groups in lysyl residues of proteins to generate pyrroles, and also because free radical oxidation is expected to generate iso[*n*]-LGs as stereoisomeric mixtures, no effort was made to achieve stereocontrol.

Synthesis of Michael Acceptor 4. The Michael acceptor for the iso[4]-LGE_2 carbon skeleton was assembled as outlined in Scheme 4. Although the reported failure of diethyl phosphonoacetone (**1**) to undergo alkylation with pentyl iodide[13] was not encouraging, the simplicity of a direct route prompted us to examine alkylation of **1** with ethyl 4-iodobutyrate. An initial attempt, reaction of the sodium enolate from **1** at room temperature for 24 h provided a low yield (<30%) of ketophosphonate **2**. However, alkylation of the sodium enolate from **1** with ethyl 4-iodobutyrate at room temperature in THF for 10 days provided ketophosphonate **2** in 55% yield. Further attempts to improve the process by using (i) a higher reaction temperature, (ii) additives such as HMPA, (iii) a potassium instead of a sodium enolate, (iv) excess NaH, or (v) excess ethyl 4-iodobutyrate did not increase the yield. It is tempting to speculate that the success of this alkylation, in contrast with that involving pentyl iodide, depends on anchimeric assistance by the ester carbonyl oxygen.

Horner−Emmons condensation of the sodium salt of **2** with isopropylidene-D-glyceraldehyde (**3**)[14] gave chiral nonracemic enones **4E** and **4Z** (4:1) in 64% yield (Scheme 4). Geometrical assignments for these isomers were made by ^{1}H NMR analysis. The pure major isomer was

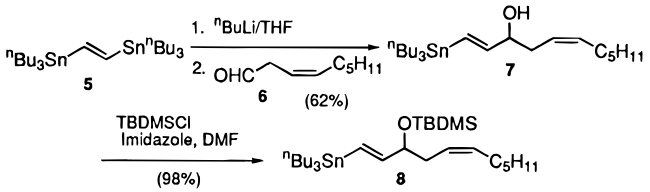
(11) This endoperoxide is also the precursor of isoprostanes that have been designated as 12-F_2-IsoP, 12-E_2-IsoP, and 12-D_2-IsoP: Taber, D. F.; Morrow, J. D.; Roberts, L. J., II. *Prostaglandins* **1997**, *53*, 63−67.

(12) (a) Salomon, R. G.; Miller, D. B.; Raychaudhri, S. R.; Avasthi, K.; Lal. K.; Levison, B. S. *J. Am. Chem. Soc.* **1984**, *106*, 8296. (b) Miller, D. B.; Raychaudhri, S. R.; Avasthi, K.; Lal. K.; Levison, B.; Salomon, R. G. *J. Org. Chem.* **1990**, *55*, 3164.

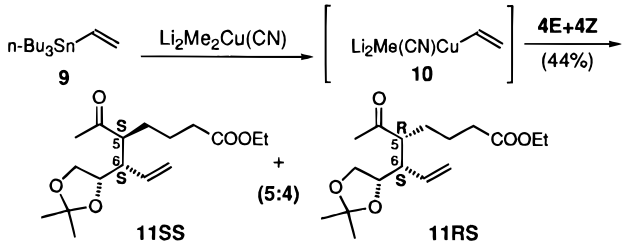
(13) Clark, D.; Kozar, L. G.; Heathcock, C. H. *Synthesis* **1975**, 635.

(14) (a) Baer, E.; Fischer, H. O. L. *J. Biol. Chem.* **1939**, *128*, 463. (b) Schmid, C. R.; Bryant, J. D. *Org. Synth.* **1993**, *72*, 6.

Scheme 5



Scheme 6

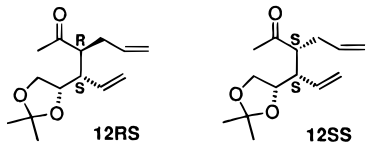


isolated by HPLC and characterized by ^{1}H NMR, ^{13}C NMR, and mass spectroscopy.

Synthesis of Vinylstannane 8. The racemic lower side chain fragment of iso[4]-LGE_2 was assembled (Scheme 5) in two steps from (*Z*)-3-nonenal (**6**) that was prepared from 1-heptyne as described previously.[15] (*Z*)-3-Nonenal (**6**), a β,γ-unsaturated aldehyde, is very sensitive owing to its proclivity to isomerize to the more stable (*E*)-2-nonenal. Therefore, crude (*Z*)-3-nonenal (**6**) was used immediately for the next step without extensive purification. Exposure of *trans*-bis(tri-*n*-butylstannyl)ethylene (**5**)[16] in THF to 1 equiv of *n*-BuLi afforded [2-(tri-*n*-butylstannyl)vinyl]lithium *in situ*. Treatment of 1.5 equiv of [2-(tri-*n*-butylstannyl)vinyl]lithium with 1 equiv of (*Z*)-3-nonenal (**6**) afforded vinylstannyl alcohol **7** in 70% yield. The alcohol **7** was converted to the corresponding known TBDMS ether **8**[15] in 98% yield (Scheme 5).

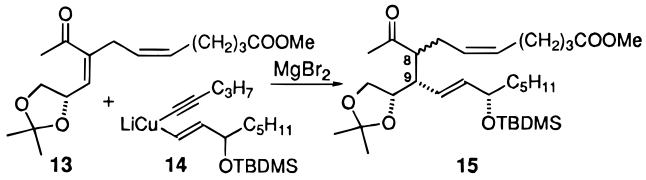
Conjugate Addition of Vinylcyanocuprate 10, a Model Side Chain Nucleophile, to γ-Alkoxy Enone 4. A novel approach for generation of mixed higher order vinylcyanocuprates by *in situ* transmetalation had been exploited for total synthesis of PGE_1 analogs.[17] The possibility of extending this methodology for the construction of the iso[4]-LGE_2 skeleton was examined. In an initial study vinylstannane **9** was used as a model for the lower side chain fragment of iso[4]-LGE_2 (Scheme 6). Conjugate addition of the higher order vinyl cyanocuprate **10** to enones **4E** + **4Z** (4:1) afforded Michael addition products **11SS** and **11RS** in 44% yield (based on enones reacted). The absolute configurations of the C-5 and C-6 centers of the conjugate addition products are specified by appropriate configurational assignment (*R* or *S*).

The ratio of diastereomers **11SS**:**11RS** was 5:4 on the basis of ^{1}H NMR analysis (*vide infra*). Thus, two asymmetric centers are created by this reaction and four diastereomeric products are possible. However, since conjugate addition of vinyl nucleophiles to γ-alkoxy enones is expected[12] to generate the *S* configuration at C-6 stereoselectively, only two products were obtained. Configurational assignments for C-5 in **11SS** and **11RS** were made by comparisons with the ^{1}H NMR spectra of compounds **12SS** and **12RS** reported previously.[12]



Conjugate Addition of a Higher Order Vinylcyanocuprate 17 to a γ-Alkoxy Enone 13. A New Synthesis of LGE_2 Precursor 15. Conjugate addition of the mixed organocuprate **14** to the γ-alkoxy enone **13** was a key step in the first total synthesis of LGE_2. It is noteworthy that this reaction required $MgBr_2$, presumably to serve as a Lewis Acid catalyst. This process is technically difficult because the requisite supersaturated solution of anhydrous $MgBr_2$ in THF readily and irreversibly deposits crystalline $MgBr_2$.

Our successful conjugate addition of the higher order vinylcyanocuprate **10** to enones **4** suggested that a higher order vinylcyanocuprate **17** might be effective as a nucleophilic lower side chain synthon for construction of the LGE_2 precursor **15**. Thus, the requisite vinylstannane **16**[18,19] and Michael acceptor **13E** + **13Z**[12] (2:1) were prepared as described previously. *In situ* transmetalation of vinylstannane **16** with $Li_2Me_2Cu(CN)$ provided the higher order cyanocuprate **17**, which upon 1,4-addition with enones **13E** + **13Z** (2:1) gave epimeric mixtures of conjugate addition products **15SS** + **15SR** and **15RS** + **15RR** in 68% yield (based on enones **13** reacted (Scheme 7). The absolute configurations at the C-8 and C-12 stereocenters in the diastereomers of **16** are specified by the designations *R* or *S*. The ratio of diastereomers (**15SS** + **15SR**):(**15RS** + **15RR**) was 1:1 on the basis of ^{1}H NMR analysis. Thus, the configurational assignments were made by comparision of ^{1}H NMR spectra with those of diastereomers of **15** reported previously.[12]



Construction of the Iso[4]-LGE_2 Skeleton 19. The higher order cyanocuprate **18** was prepared by transmetalation of vinylstannane **8** with $Li_2Me_2Cu(CN)$.[17] Addition of a mixture of isomeric enones **4E** and **4Z** (4:1) to the higher order vinylcyanocuprate **18** provided a mixture of diastereomeric conjugate addition products **19**. The diastereomeric conjugate addition products were separated by flash column chromatography to provide C5,C6-threo (**19SS** + **19SR**) and C5,C6-erthyro (**19RR** + **19RS**) isomers in the ratio of 45:55 (Scheme 8). The absolute configurations of the C-5 and C-9 stereocenters of the conjugate addition products are specified by appropriate configurational assignments (*R* or *S*). The overall yield of conjugate addition products was 56% on the basis of the enone reacted. HPLC analysis of erythro isomers (**19RR** + **19RS**) showed two close peaks (retention times 9.1 and 9.4 min) which were not readily separable. The stereochemical assignments are based on the similarity in the C5 proton resonance chemical shifts with those of conjugate addition products **11SS** (threo)

(15) Kobayashi, Y.; Shimazaki, T.; Taguchi, H.; Sato, F., *J. Org. Chem.* **1990**, *55*, 5324.

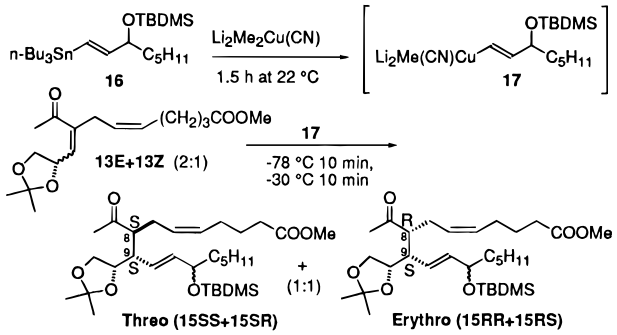
(16) Bottaro, J. C.; Hanson, R. N.; Seitz, D. E. *J. Org. Chem.* **1978**, *46*, 5221.

(17) Behling, J. R.; Babiak, K. A.; Ng, J. S.; Campbell, A. L.; Moretti, R.; Koerner, M.; Lipshutz, B. H. *J. Am. Chem. Soc.* **1988**, *110*, 2641.

(18) Johnson, C. R.; Penning, T. D. *J. Am. Chem. Soc.* **1988**, *110*, 4726.

(19) Chen, S.-M. L.; Schaub, R. E.; Grudzinskas, C. V. *J. Org. Chem.* **1978**, *43*, 3450.

Scheme 7



Scheme 8

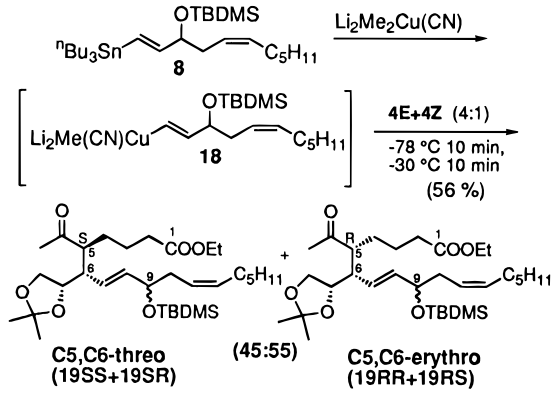
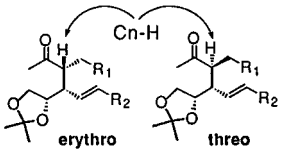


Table 1. ^{1}H NMR Chemical Shift Comparisions of the Cn-H Resonances for a Series of Erythro and Threo Diastereomers[a]



Entry	R_1	R_2	n	Cn-H erythro	Cn-H threo	Cn-H Δe-t
1	-CH=CH_2	H	4	3.10	2.84	0.26
2	-CH=CH_2		4	3.06	2.71	0.35
3	-(Z)-CH=CH-$(CH_2)_3CO_2Me$		8	2.99	2.58	0.41
4	$(CH_2)_2CO_2Et$	H	5	2.97	2.60	0.37
5	$(CH_2)_2CO_2Et$		5	2.94	2.64	0.30
6	$(CH_2)_2CO_2H$		5	2.94	2.64	0.30

[a] Chemical shift values for entries 1−3[12] were obtained from 200 MHz ^{1}H NMR spectra, and for the entries 4−6 the values were obtained from 300 MHz ^{1}H NMR spectra.

and **11RS** (erythro) and those reported previously for LGE_2 precursor **15** (see Table 1).[12] Thus, the conjugate addition products having an 5,6-erythro side chain arrangement, i.e., **19RR** and **19RS**, are readily distinguished from the corresponding threo products, i.e., **19SS** and **19SR**, by chromatography and ^{1}H NMR spectroscopy. The C5,C6-erythro products are less polar (R_f = 0.15) than the C5,C6-threo products (R_f = 0.11). Therefore, the four diastereomeric products were readily separated into threo and erythro pairs of C9 epimers.

Saponification of Ethyl Ester 19. Saponification of ethyl ester **19** with sodium hydroxide in water/methanol/THF (2:5:3, v/v/v) afforded the acid **20** (Scheme 9). It is

Scheme 9

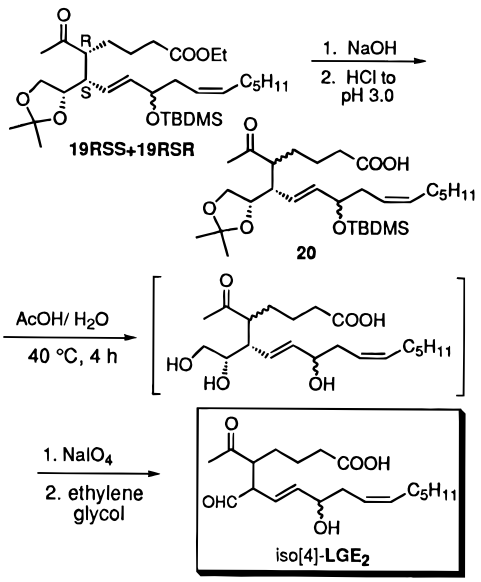
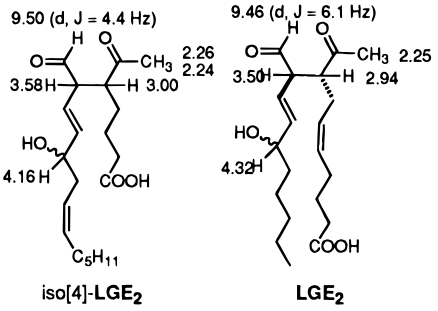


Chart 1

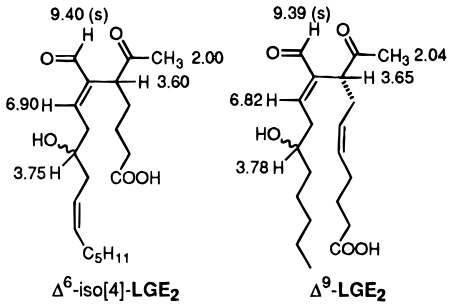


interesting to note that saponification of either C5,C6-threo or C5,C6-erythro isomers of **19** leads to same mixture of diastereomeric carboxylic acids **20** due to epimerization at the 5-position during ester hydrolysis.[12] The ratio of C5,C6-erythro to C5,C6-threo isomers was 6:4 on the basis of ^{1}H NMR analysis. The chemical shift position of the C5 proton resonance for the C5,C6-erythro diastereomers occurs at δ 2.94 while that for the C5,C6-threo diastereomers occurs at δ 2.66.

Generation of Iso[4]-LGE_2 and Methoxime Derivatives. Deprotection of the hydroxyl groups in **20** by treatment with acetic acid−water (2:1 v/v) at 40 °C for 4 h, followed by oxidative cleavage of the resulting vicinal diol with sodium periodate, afforded iso[4]-LGE_2 as a mixture of diastereomers (Scheme 9). Thus, the ^{1}HNMR spectrum shows a doublet at δ 9.50 (J = 4.4 Hz) for the aldehyde hydrogen. This is similar to the corresponding resonance at δ 9.46 (J = 6.1) in the spectrum of LGE_2 (Chart 1). The three methine resonances at δ 3.00, 3.56, and 4.16 in the spectrum of iso[4]-LGE_2 closely match the corresponding resonances a δ 2.94, 3.50, and 4.32 in the spectrum of LGE_2. It is important to note that, at most, only a trace of the α,β-unsaturated aldehyde isomer Δ^6-iso[4]-LGE_2, analogous to Δ^9-LGE_2, is present in this product. Thus, only a tiny singlet at δ 9.40 and multiplet at δ 6.90 appear in the spectrum of the product which can be assigned to aldehyde and β-vinyl hydrogens in Δ^6-iso[4]-LGE_2 (*vide infra*).

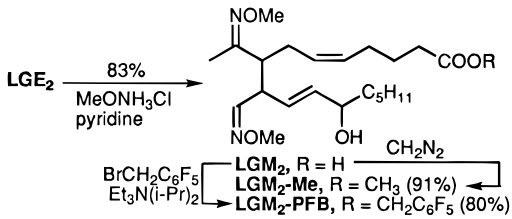
Synthetic iso[4]-LGE_2 provides authentic samples for comparison with samples of this putative natural product isolated from biological sources. However, iso[4]-LGE_2 is a chemically sensitive vinylogous β-hydroxy aldehyde.

Chart 2



Δ^6-iso[4]-**LGE$_2$** Δ^9-**LGE$_2$**

Scheme 10



As for LGE_2, isolation and purification of iso[4]-LGE_2 is complicated by a proclivity toward dehydration as well isomerization of the β,γ-unsaturation into conjugation with the aldehyde carbonyl. Thus, a sample stored in $CDCl_3$ at 3 °C isomerized cleanly to the more stable α,β-unsaturated isomer, Δ^6-iso[4]-LGE_2, within a few days as evidenced by a singlet at δ 9.40 and multiplet at δ 6.90 owing to the aldehyde and β-vinyl hydrogens. Δ^9-LGE_2 shows corresponding resonances at δ 9.39 and 6.82 (Chart 2).

PGD_2 undergoes a variety of similar dehydration and rearrangement reactions forming PGJ_2, Δ^{12}-PGJ_2, $\Delta^{12,14}$-PGD_2, and $\Delta^{12,14}$-PGJ_2. However, PGD_2 is converted into a stable methoxime derivative upon treatment with methoxylamine hydrochloride in pyridine.[20] Furthermore, methoxime derivatives of PGs having sensitive β-ketol functionality are suitable for GC analysis, and pentafluorobenzyl (PFB) esters of methoxime trimethylsilyl (TMS) ethers of these PGs have been exploited for GC-MS analysis.[21]

Reaction of LGE_2 with methoxylamine hydrochloride in pyridine proceeds cleanly to give the bismethoxime LGM_2 (Scheme 10). LGM_2 is stable enough to be purified by flash chromatography or by HPLC. LGM_2 is a mixture of syn−anti methoxime stereoisomers which were inseparable chromatographically. The methyl ester of LGM_2 was prepared by treatment with diazomethane, and the pentafluorobenzyl (PFB) ester was prepared by treatment with pentafluorobenzyl bromide and diisopropylamine (Scheme 10). Both the methyl and PFB esters of LGM_2 were separable by HPLC into major and minor diastereomers. For mass spectral analysis, the hydroxyl group in LGM_2−PFB was silylated by treatment with *N*,*N*′-bis(trimethylsilyl)trifluoroacetamide to provide LGM_2−TMS-PFB (*vide infra*). Similar derivatization of iso[4]-LGE_2 provided iso[4]-LGM_2−TMS-PFB. Methoximation could also be achieved in aqueous solution, an especially convenient procedure for derivitization of biological samples.

Mass Spectral Characterization of LGE_2 and Iso[4]-LGE_2. All of the bismethoxime esters give strong parent ion peaks and characteristic fragments in their mass spectra. Owing to a strong proclivity toward dehydration, the EI mass spectrum of LGE_2 methyl ester shows only a tiny parent ion.[22] In contrast, the parent ion of LGM_2 methyl ester at *m*/*z* 424.29 is 12.5% of the base peak at 226. Iso[4]-LGM_2−TMS-PFB was characterized and compared with LGM_2−TMS-PFB by electron impact (EI, 24 eV) high-resolution mass spectrometry (HRMS). Characteristic mass spectral fragments of iso[4]-LGM_2−TMS-PFB and LGM_2−TMS-PFB are summarized in Charts 3 and 4, respectively.

Chart 3. Characteristic Ions from Iso[4]-LGM_2−TMS-PFB

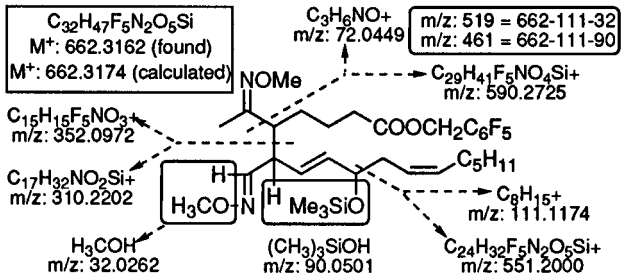
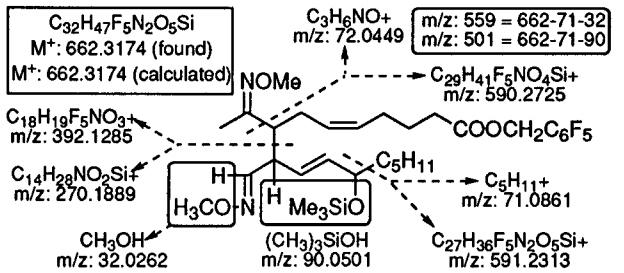


Chart 4. Characteristic Ions from LGM_2−TMS-PFB



Several peaks are diagnostic for LGs and isoLGs as a family, but do not distinguish between different members of the family. Thus, both iso[4]-LGM_2−TMS-PFB and LGM_2−TMS-PFB show intense $C_3H_6NO^+$ ion peaks at *m*/*z* 72 which correspond to the methoxime derivative of an acylium ion (*m*/*z* 43) that is the base peak in the mass spectrum of LGE_2.[22] Parent ions at *m*/*z* 662 and ions at *m*/*z* 590, corresponding to loss of the methoxime of an acyl group, are also present in the mass spectra of both the LG and isoLG bismethoxime TMS-PFB derivatives.

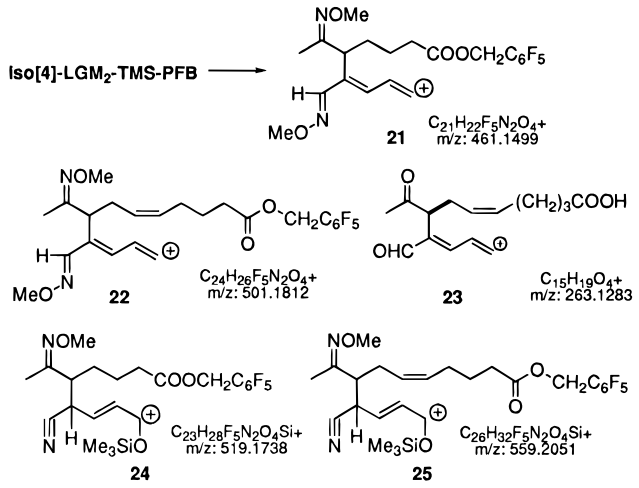
Especially characteristic of iso[4]-LGM_2−TMS-PFB are ions at *m*/*z* 310 and 352 corresponding to the lower and upper halves of the molecule. These ions are replaced by those at *m*/*z* 270 and 392 in the mass spectrum of LGM_2-TMS-PFB. Loss of trimethylsilanol and carbons 10−17 produces the *m*/*z* 461 ion **21** from iso[4]-LGM_2−TMS-PFB. An analogous fragmentation of LGM_2−TMS-PFB, i.e., loss of trimethylsilanol and carbons 13−17, produces the *m*/*z* 501 ion **22**, while a corresponding loss of water and carbons 13−17 produces the *m*/*z* 263 ion **23** that is prominant in the mass spectrum of LGE_2. Similarly, loss of methanol and carbons 10−17 produces the *m*/*z* 519 ion **24** from iso[4]-LGM_2−TMS-PFB while a corresponding loss of methanol and carbons 13−17 from LGM_2−TMS-PFB produces the *m*/*z* 559 ion **25**.

GC-MS experiments using synthetic iso[4]-LGM_2−TMS-PFB to detect and confirm the production of iso[4]-

(20) Ito, S.; Tanaka, T.; Hayashi, H.; Hayaishi, O. *Eicosanoids* **1988**, *1*, 111.

(21) (a) Fitzpatrick, F. A. *Anal. Chem.* **1978**, *50*, 47. (b) Berens, M. E.; Salmon, S. E.; Davis, T. P. *J. Chromatogr.* **1984**, *307*, 251.

(22) Salomon, R. G.; Miller, D. B. In *Advances in Prostaglandin, Thromboxane, and Leucotriene Research 15*; Hayaishi, O., Yamamoto, S., Eds.; Raven Press: New York, 1985; pp 323−326.



LGE_2 during *in vitro* free radical oxidation of arachidonic acid are planned. An immunoassay for detecting iso[4]-LGE_2−protein adducts *in vitro* and *in vivo* is being developed in our laboratories using antibodies raised against protein adducts of synthetic iso[4]-LGE_2. These and other applications of authentic samples of iso[4]-LGE_2 to the study of lipid oxidation will be reported elsewhere.

Experimental Procedures

General Methods. ^{1}H NMR and ^{13}C NMR spectra were recorded at 300 and 75 MHz, respectively, and are reported as described previously.[12] High-resolution mass spectra, solvent purification, and chromatography were performed as usual.[12] All reactions conducted in an inert atmosphere were in argon unless otherwise specified. Ethyl 4-iodobutyrate,[23] isopropylidene-D-glyceraldehyde[14] (**3**), 3(*Z*)-nonenal[15] (**6**), *trans*-bis(tributylstannyl)ethylene[16] (**5**), 3-[(*tert*-butyldimethylsilyl)oxy]oct-1-yne,[18,24] 3-[(*tert*-butyldimethylsilyl)oxy]-1-(tributylstannyl)-1(*E*)-octene[19] (**16**), and methyl 8-acetyl-10(*S*),11-(isopropylidenedioxy)-5,8-undecadienoate[12] (**13**) were prepared by literature procedures.

Ethyl 5-(Diethylphosphono)-6-oxoheptanoate (2). Alkyl phosphonate **2** was prepared by alkylation of diethyl phosphonoacetone (**1**).[12] To a magnetically stirred suspension of sodium hydride (2.80 g, 95% pure, 111 mmol) in anhydrous THF (222 mL) was added diethyl phosphonoacetone (21.55 g, 111 mmol) in THF (22.2 mL) at 0 °C. The mixture was allowed to stir at room temperature for 2 h to form a clear yellow solution. Ethyl 4-iodobutyrate (32.24 g, 133.2 mmol) was added, and the solution was stirred in the dark for 10 days at room temperature. The reaction was followed by TLC and there was a significant amount of unreacted starting phosphonate even after 10 days. The solvent was removed by rotary evaporation, and water (160 mL) was added to the resulting dark yellow residue. The aqueous mixture was extracted with ethyl acetate, and the extracts were washed with brine, dried (anhydrous $MgSO_4$), filtered, and concentrated under reduced pressure to afford a clear yellow oil. This oil was distilled under reduced pressure to provide starting phosphonate **1** (74−77 °C at 0.04 mmHg, 6.5 g) and the *β*-ketophosphonate **2** (127−129 °C at 0.04 mmHg, 13.1 g, 55% based on phosphonate **1** reacted): ^{1}H NMR ($CDCl_3$, 300 MHz) *δ* 4.10−3.90 (apparent quintet, *J* = 7.1 Hz, 6H), 3.15−2.95 (ddd, H, *J* = 24.7, 10.5, 3.6 Hz), 2.26 (s, 3H), 2.25−2.10 (t, 2H, *J* = 7.3 Hz), 2.05−1.80 (m, H), 1.75−1.60 (m, H), 1.54−1.46 (m, 2H), 1.30−1.23 (t, 6H, *J* = 7.1 Hz), 1.20−1.15 (t, 3H, *J* = 7.1 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) *δ* 203 (+), 202.96 (+), 172.47 (+), 62.37 (+), 62.28 (+), 62.21 (+), 62.12 (+), 59.95 (+), 53.84 and 52.19 (d, *J* = 124.9 Hz, C-5 split by ^{31}P, −), 33.43 (+), 30.76 (−), 25.46 (+), 25.40 (+), 23.42 (+), 23.22 (+), 15.97 (−), 15.90 (−), 15.81 (−); HRMS (20 eV) *m*/*z* calcd for $C_{13}H_{25}O_6P$ 308.1388, *m*/*z* (relative intensity) found 308.1371 (1), 165 (100).

Ethyl 5-Acetyl-7(*S*),8-(isopropylidenedioxy)-5-octenoates (4E and 4Z). A magnetically stirred solution of sodium hydride (570 mg, 23.75 mmol, 1.25 equiv) in anhydrous THF (37.5 mL) was cooled to −5 °C. The *β*-ketophosphonate **2** (5.87 g, 19.0 mmol) in anhydrous THF (37.5 mL) was added via an airtight syringe over 10 min. Stirring was continued at this temperature for 4 h. Then freshly prepared isopropylidene-D-glyceraldehyde (**3**) (3.79 g, 21.85 mmol, 1.1 equiv, 80% purity) in anhydrous THF (9.4 mL) was added over 5 min. The solution was allowed to warm to room temperature, and stirring was continued for an additional 12 h. Then the solvent was removed by rotary evaporation, and water (90 mL) was added to the resulting brown oily, gummy residue. The aqueous mixture was extracted with diethyl ether, the extracts were washed with water, dried (anhydrous $MgSO_4$), and filtered, and then the solvent was removed under reduced pressure to afford a clear yellow oil (5.3 g). This oil was flash chromatographed in two batches, each approximately 2.6 g, on a 12-cm column packed with a 15-cm bed of silica gel. The column was eluted with 22% ethyl acetate in hexanes (v/v). Forty-five 50 mL fractions were collected. The mixtures of enones (**4E** and **4Z**, R_f = 0.28) were eluted in fractions 23−40 as clear oil (3.45 g, 64% based on *β*-ketophosphonate **2**). The **4Z** and **4E** isomers (1:4, respectively) were used without separation for the next reaction. Mixture of **4Z** (* denotes minor isomer) and **4E** (major) isomers: ^{1}H NMR ($CDCl_3$, 300 MHz) *δ* 6.50 (d, H, *J* = 8.0 Hz), 5.72* (d, H, *J* = 8.0 Hz), 4.92 (apparent q, *J* = 7.60 Hz), 4.82* (apparent q, *J* = 7.60 Hz), 4.26* (dd, H, *J* = 8.0, 6.6 Hz), 4.20 (dd, H, *J* = 8.0, 6.6 Hz), 4.10 (q, 2H, *J* = 7 Hz), 4.09* (q, 2H, *J* = 7 Hz), 3.63 (dd, 2H, *J* = 8.1, 7.4 Hz), 3.53* (dd, 2H, *J* = 8.1, 7.4 Hz), 2.32 (s, 3H), 2.30 (t, 2H, *J* = 8 Hz), 2.25* (s, 3H), 2.20−1.50 (m, 2H), 2.45−2.20 (m, 3H), 1.82−1.60* (m, 2H), 1.46 (s, 3H), 1.40 (s, 3H), 1.39* (s, 3H), 1.32* (s, 3H), 1.24 (t, 3H, *J* = 8 Hz), 1.23* (t, 3H, *J* = 8 Hz); ^{13}C NMR ($CDCl_3$, 75.4 MHz) *δ* 202.17* (+), 198.93 (+), 173.22 (+), 173.12* (+), 143.36 (+), 141.30* (+), 140.11 (−), 137.57* (−), 110.03 (+), 109.80* (+), 73.88* (−), 72.66 (−), 69.80* (+), 69.04 (+), 60.34 (+), 60.02* (+), 33.78 (+), 33.20* (+), 26.68 (−), 26.60* (−), 25.70 (−), 25.51* (−), 25.31 (−), 25.10 (−), 24.80 (+), 24.50* (+), 23.61* (+), 14.21 (−), 14.20* (−). Pure major isomer **4E** was isolated by the HPLC using 22% ethyl acetate in hexane as eluant with a Whatman Patrisil 10, M9 column (9.4 mm i.d. × 50 cm) at a flow rate of 3 mL/min (retention time = 19 min). **4E** (major isomer): HRMS (20 eV) *m*/*z* calcd for $C_{14}H_{21}O_5^+$ ($M^+ - CH_3$) 269.1389, *m*/*z* (relative intensity) found 269.1374 (1), 209 (100).

1-(Tri-*n*-butylstannyl)-1(*E*),5(*Z*)-undecadien-3-ol (7). The required aldehyde, 3(*Z*)-nonenal **21** (1.23 g, 9.33 mmol), was freshly prepared from 1,1-diethoxy-3(*Z*)-nonene (2.0 g, 9.33 mmol) according to the reported procedure.[15] To a solution of *trans*-bis(tri-*n*-butylstannyl)ethylene[16] (**5**, 9.72 g, 16.1 mmol) in THF (25 mL) was slowly added *n*-BuLi (11 mL, 1.4 M in hexane, 15.4 mmol). After the solution was stirred for 1 h at −60 °C, 3(*Z*)-nonenal (**6**, 1.23 g) was added. The solution was stirred for 1 h at 0 °C. The reaction was then quenched with saturated ammonium chloride solution (10 mL). The mixture was extracted with hexanes. The extract was dried (anhydrous $MgSO_4$) and concentrated under reduced pressure to afford a colorless residue (12.5 g). This residue was flash chromatographed on a 12-cm column packed with a 15-cm bed of silica gel. The column was eluted with with hexanes, which removes nonpolar side products Bu_4Sn and $Bu_3SnCH{=}CH_2$, and then with 5% ethyl acetate in hexanes to provide vinylstannane alcohol **7** (R_f = 0.17, 2.98 g). The alcohol **7** was further purified by preparative HPLC, using a Gilson autoinjection-collection system with 4% ethyl acetate/hexane using a Whatman Patrisil 10, M9 column (9.4 mm i.d. × 50 cm) at a flow rate of 4 mL/min (retention time = 17 min) to provide pure 1-(tri-*n*-butylstannyl)-1(*E*),5(*Z*)-undecadien-3-ol, **7** (2.64 g, 70% based on acetal): ^{1}H NMR ($CDCl_3$, 300 MHz) *δ* 6.25 (d, H, *J* = 19.2 Hz), 6.00 (dd, H, *J* = 19.2, 5.0 Hz), 5.60−5.52 (m, H), 5.42−5.28 (m, H), 4.15−4.05 (m, H), 2.35−2.20 (t, *J* = 6.6 Hz, 2H),

(23) Abraham, E. P.; Smith, J. C. *J. Am. Chem. Soc.* **1939**, *61*, 1605.
(24) Corey, E. J.; Venkateswaralu, A. *J. Am. Chem. Soc.* **1972**, *94*, 6190.

2.10−1.95 (q, J = 6.8 Hz, 2H), 1.70−1.65 (broad s, 1H), 1.60−1.20 (m, 24H), 0.9−0.7 ([t, J = 7.2 Hz, 9H] and 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 150.29 (−), 132.64 (−), 127.19 (−), 124.52 (−), 74.54 (−), 34.93 (+), 31.38 (+), 29.20 (+), 28.92 (+), 27.27 (+), 27.09 (+), 22.41 (+), 13.84 (−), 13.47 (−), 9.2 (+); HRMS (20 eV) *m/z* calcd for $C_{19}H_{37}O^{120}Sn$ ($M^+ - C_4H_9$) 401.1867; *m/z* (relative intensity) found 401.1889 (20), 177 (100), 137 (40), 120 (37).

3-[(*tert*-Butyldimethylsilyl)oxy]-1-(tri-*n*-butylstannyl)-1(*E*),5(*Z*)-undecadiene (8). Alcohol **7** was converted to the corresponding TBDMS ether using Corey's methodology.[24] A solution of 1-(tributylstannyl)-1(*E*),5(*Z*)-undecadien-3-ol (**7**, 1.29 g, 2.82 mmol), *tert*-butyldimethylsilyl chloride (0.686 g, 4.55 mmol), and imidazole (0.62 g, 9.12 mmol) in dry DMF (8 mL) was stirred at room temperature for 12 h and then poured into a mixture of hexanes and saturated aqueous $NaHCO_3$. The aqueous layer was extracted with hexanes. The extract was dried (anhydrous $MgSO_4$) and concentrated under reduced pressure to afford an oily residue. This oily residue was purified by flash chromatography on a 12-cm column packed with a 15-cm bed of silica gel. The column was eluted with with hexanes to afford a clean colorless oil, the desired silyl ether, 3-[(*tert*-butyldimethylsilyl)oxy]-(tri-*n*-butylstannyl)-1(*E*),5(*Z*)-undecadiene (**8**, R_f = 0.37, 1.58 g, 98%). The other minor product (<2%) formed in this reaction was presumably 3-[(*tert*-butyldimethylsilyl)oxy]-1,5(*Z*)-undecadiene. ^{1}H NMR and ^{13}C NMR spectra of TBDMS ether **8** were consistent with those reported.[15] **8**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.08 (d, J = 19.0 Hz, 1H), 5.95 (dd, J = 19.0, 5.2 Hz), 5.50−5.30 (m, 2H), 4.10−4.00 (m, 1H), 2.30−2.20 (apparent q, J = 6.6 Hz, 2H), 2.05−1.95 (q, J = 6.6 Hz), 1.60−1.20 (m, 24H), 0.95−0.70 (m, 21H), 0.02−0.01 (2s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 151.15 (−), 131.33 (−), 126.36 (−), 125.47 (−), 76.48 (+), 36.07 (+), 31.48 (+), 29.30 (+), 29.02 (+), 27.35 (+), 27.15 (+), 25.78 (−), 22.49 (+), 13.94 (−), 13.58 (−), 9.29 (+),-4.58 (−), −4.92 (−); HRMS (20 eV) *m/z* calcd for $C_{25}H_{51}OSi^{120}Sn$ ($M^+ - C_4H_9$) 515.2731, *m/z* (relative intensity) found 515.2714 (6), 171 (100).

4-(3,3-Dimethyl-2,4-dioxolanyl)-3-prop-2-enylhex-5-en-2-ones 11. The vinylcuprate reagent **10** was generated *in situ* by transmetalation of vinylstannane **9** with higher order dimethyl cyanocuprate as described previously.[17] Copper cyanide (41 mg, 0.45 mmol, flame dried under argon) in THF (0.5 mL) was treated with methyllithium (0.99 mmol, 0.8 mL of 1.24 M, 2.20 equiv) at 0 °C. The cooling bath was removed, and vinylstannane **9** (161 mg, 0.51 mmol, 1 equiv) in THF (1 mL) was added. After 1.5 h at room temperature, the reaction was cooled to −78 °C and enone **4** (*Z*/*E* 1:4, 65 mg, 0.23 mmol, 0.5 equiv) in THF (0.5 mL) was added rapidly via a syringe. After the mixture was stirred at −78 °C for 10 min and at −30 °C for 10 min, the reaction was quenched by addition of saturated aqueous ammonium chloride and ammonium hydroxide (9:1, v/v, 2 mL). The resulting mixture were then extracted with diethyl ether. The extract was successively washed with water and brine, dried (anhydrous $MgSO_4$), filtered, and concentrated by rotary evaporation. The crude product (240 mg) was passed through a silica gel plug and then purified by HPLC with 20% ethyl acetate in hexane as eluant to afford the **11SS** and **11RS** (16 mg, 44% based on enone reacted). The ratio of erthro (9 mg, retention time = 17 min) and threo (7 mg, retention time = 21 min) isomers was 4:5. The unreacted enone **4** recovered was almost exclusively the *E* isomer. **C5−C6 erythro isomer (11RS)**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.72−5.58 (m, 1H), 5.15−5.00 (m, 2H), 4.09 (q, J = 7 Hz, 2H), 4.94−3.86 (m, 2H), 3.58−3.48 (m, 1H), 3.02−2.92 (m, 1H), 2.35−2.22 (m, 3H), 2.20 (s, 3H), 1.70−1.60 (m, 4H), 1.39 and 1.31 (2s, 6H), 1.22 (t, 3H); HRMS (20 eV) *m/z* calcd for $C_{17}H_{28}O_5$ 312.1937, *m/z* (relative intensity) found 312.1920 (1), 140 (100). **C5−C6 threo isomer (11SS)**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.45−5.30 (m, 1H), 5.12−5.00 (m, 2H), 4.10 (q, J = 7 Hz, 2H), 4.00−3.85 (m, 2H), 3.65−3.55 (m, 1H), 2.65−2.55 (m, 1H), 2.52−2.42 (m, 1H), 2.28−2.20 (t, 2H), 2.16 (s, 3H), 1.60−1.40 (m, 4H), 1.36 and 1.30 (2s, 6H), 1.22 (t, 3H); HRMS (20 eV) *m/z* calcd for $C_{17}H_{28}O_5$ 312.1937, *m/z* (relative intensity) found 312.1934 (0.2), 101 (100).

3-[(*tert*-Butyldimethylsilyl)oxy]-1-(tributylstannyl)-1(*E*)-octene (16). 1-Octyn-3-ol was converted to the corresponding TBDMS ether[18] using Corey's methodology.[24] A solution of 1-octyn-3-ol (11.7 g, 92.4 mmol), *tert*-butyldimethylsilyl chloride (25.0 g, 166 mmol), and imidazole (12.6 g, 185 mmol) in dry DMF (170 mL) was stirred at room temperature for 8 h and then poured into a mixture of hexanes and saturated aqueous $NaHCO_3$. The aqueous layer was extracted with hexanes. The extract was dried (anhydrous $MgSO_4$) and concentrated under reduced pressure to afford an oily residue (33 g) that was distilled under reduced pressure to afford the desired 3-[(*tert*-butyldimethylsilyl)oxy]-1-octyne (22.2 g, 100%, bp 44.5−45 °C at 0.3 mmHg): ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.36 (td, J = 6.5, 2.1 Hz, 1H), 2.34 (d, J = 2.1 Hz, 1H), 1.62 (m, 2H), 1.45−1.20 (6H), 0.90−0.85 (12H), 0.10 (s, 3H), 0.07 (s, 3H). Without any further purification, 3-[(*tert*-butyldimethylsilyl)oxy]-1-octyne was used for the next step. To a stirred mixture of 3-[(*tert*-butyldimethylsilyl)oxy]-1-octyne (10.0 g, 41.6 mmol) and AIBN (75 mg) was added tri-*n*-butylstannane (17.4 g, 59.8 mmol) with an airtight syringe under an argon atmosphere. The mixture was heated at 130 °C, stirred for 2 h, and then cooled to room temperature. The excess tri-*n*-butylstannane was removed by distillation under reduced pressure (53−75 °C at 0.05 mmHg). The product mixture was further distilled to afford 3-[(*tert*-butyldimethylsilyl)oxy]-1-(tributylstannyl)-1(*E*)-octene (**16E**, 20.0 g, 90%, bp 138−148° C at 0.05 mmHg, 81% isomerically pure) as a clear oil along with (*Z*)-vinylstannane and the 2-tri-*n*-butylstannyl regioisomer. The isomeric purity of the (*E*)-vinylstannane (81%) was calculated from the integration of the vinyl proton resonances in the ^{1}H NMR spectra as previously reported.[18] The ^{1}H NMR spectrum of vinylstannanes **16** was consistent with that reported.[18]

A New Synthesis of LGE$_2$ Precursor Methyl 8-Acetyl-9-(3,3-dimethyl-2,4-dioxolanyl)-12-(1,1-dimethyl-1-silaethoxy)heptadeca-5,10-dienoate (15). Copper cyanide (63 mg, 0.70 mmol, flame dried under argon) in THF (1.0 mL) was treated with methyllithium (1.50 mmol, 1.27 mL of 1.18 M, 2.14 equiv) at 0 °C. The cooling bath was removed, and vinylstannane **16** (459 mg, 81% pure, 0.70 mmol, 1 equiv) in THF (1.0 mL) was added. After 1.5 h at room temperature, the mixture was cooled to −78 °C and enone **13** (*E*/*Z* = 2:1, 155 mg, 0.50 mmol, 0.71 equiv) in THF (1.0 mL) was added rapidly via a syringe. After the solution was stirred at −78 °C for 10 min and at −30 °C for 10 min, the reaction was quenched by addition of saturated aqueous ammonium chloride and ammonium hydroxide (9:1, v/v, 2 mL). The resulting mixture was then extracted with diethyl ether. The extract was successively washed with water and brine, dried (anhydrous $MgSO_4$), filtered, and concentrated by rotary evaporation. TLC analysis (20% ethyl acetate in hexanes) of the crude product showed four spots corresponding to tri-*n*-butylstannylmethane (R_f = 0.65), a diasteromeric mixture of conjugate addition products **15** (R_f = 0.34 and 0.32) and unreacted enone **13** (R_f = 0.13). The unreacted enone recovered was almost exclusively as the *E* isomer. The crude product (574 mg) was purified by flash chromatography on a 6 cm column packed with a 15 cm bed of silica gel. The column was eluted with 20% ethyl acetate in hexanes to afford the starting enone **13** (19 mg) and the diasteromeric conjugate addition products **15** (160 mg, 68% based on enone reacted). The ratio of erythro to threo isomers was 1:1. ^{1}H NMR spectra of the erythro and threo isomers were consistent with those reported previously.[12]

Construction of the Iso[4]-LGE$_2$ Skeleton: Ethyl 5-Acetyl-6-(3,3-dimethyl-2,4-dioxolanyl)-9-(1,1-dimethyl-1-silaethoxy)heptadeca-7,11-dienoate (19). Copper cyanide (94.5 mg, 1.06 mmol, flame dried under argon) in THF (1.5 mL) was treated with methyllithium (2.26 mmol, 2.37 mL of 0.95 M, 2.14 equiv) at 0 °C. The cooling bath was removed, and vinylstannane **8** (603 mg, 1.06 mmol, 1 equiv) in THF (1.5 mL) was added. After 1.5 h at room temperature, the reaction flask was cooled to −78 °C with a dry ice−acetone bath and enone **4** (*Z*/*E* = 1:4, 300 mg, 1.06 mmol, 1 equiv) in THF (1.5 mL) was added rapidly via a syringe. After the solution was stirred at −78 °C for 10 min and at −30 °C for 10 min, the reaction was quenched by addition of saturated aqueous ammonium chloride and ammonium hydroxide (9:1, v/v, 2 mL). The resulting mixture was then extracted with diethyl ether.

The extract was successively washed with water and brine, dried (anhydrous $MgSO_4$), filtered, and concentrated by rotary evaporation. TLC analysis (10% ethyl acetate in hexanes) of the crude product showed four spots corresponding to (tri-*n*-butylstannyl)methane ($R_f = 0.72$), a diasteromeric mixture of conjugate addition products **19** (R_f = 0.15 and 0.11), and unreacted enone **4** (R_f = 0.05). The unreacted enone **4** recovered was almost exclusively the *E* isomer. The crude product (856 mg) was purified by flash chromatography on a 5 cm column packed with a 25 cm bed of silica gel. The column was eluted with 15% ethyl acetate in hexanes to afford the threo isomers **19SS** and **19SR** (84 mg) and erythro isomers **19RS** and **19RR** (104 mg). The ratio of erythro to threo isomers was 55:45. The overall yield (56%) of conjugate addition products was calculated on the basis of enone reacted (188 mg). **Erythro isomers (19RR and 19RS)**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.47−5.26 (m, 4H), 4.09 (q, $J = 7$ Hz, 2H), 4.10−4.00 (m, 1H), 3.91−3.83 (m, 2H), 3.52−3.46 (m, 1H), 2.97−2.90 (m, 1H), 2.32−2.05 (m, 5H), 2.19 (s, 3H), 2.00−1.93 (q, $J = 6$ Hz), 1.70−1.15 (m, 19H), 0.95−0.85 (apparent t, 3H), 0.857 (s, 9H), 0.03−0.00 (m, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 212.37, 173.61, 138.28, 132.28, 126.20, 125.87 and 125.39, 109.76, 77.85, 76.32, 73.51, 73.28, 69.51, 69.46, 60.69, 52.50, 52.34, 50.15, 49.97, 36.75, 34.63, 34.58, 32.39, 31.94, 29.90, 29.71, 27.86, 27.32, 26.22, 26.00, 23.35, 23.12, 22.96, 18.61, 14.63, 14.46, −4.11, −4.18, −4.39, and −4.43; HRMS (20 eV) *m/z* calcd for $C_{31}H_{55}O_6Si$ ($M^+ - CH_3$) 551.3768, *m/z* (relative intensity) found 551.3804 (4), 239 (100). **Threo isomers (19SR and 19SS)**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.54−5.12 (m, 4H), 4.08 (q, $J = 7$ Hz, 2H), 4.10−4.00 (m, 1H), 3.95−3.80 (m, 2H), 3.60−3.50 (m, 1H), 2.70−2.58 (m, 1H), 2.50−2.38 (m, 1H), 2.30−2.05 (m, 4H) 2.14 and 2.13 (two apparent s, 3H), 1.97 (q, J = 6.5 Hz, 2H), 1.70−1.20 (m, 19H), 0.95−0.85 (apparent t, 3H), 0.853 (s, 9H), 0.00−0.08 (m, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 211.31, 211.03, 173.60, 138.25, 132.66, 132.44, 125.96, 125.70, 125.26, 125.20, 109.96, 77.85, 77.54, 73.13 and 73.02, 69.08, 60.65, 54.99, 54.82, 50.09, 36.71, 36.65, 34.69, 31.93, 30.49, 30.38, 29.70, 28.68, 28.12, 27.85, 26.94, 26.85, 26.20, 25.98, 23.45, 23.38, 22.96, 18.61, 14.63, 14.46, −4.18, −4.23 and −4.40; HRMS (20 eV) *m/z* calcd for $C_{31}H_{55}O_6Si$ ($M^+ - CH_3$) 551.3768, *m/z* (relative intensity) found 551.3752 (1), 239 (100).

Hydrolysis of Ethyl Ester 19. To a solution of erythro ethyl esters **19RR** and **19RS** (23 mg, 0.042 mmol) in water/methanol/THF (2:5:3, v/v/v, 0.5 mL) was added a solution of sodium hydroxide (8 mg, 0.2 mmol) in water/methanol/THF (2:5:3, v/v/v, 0.5 mL). The contents were stirred at room temperature for 90 min whereupon TLC analysis with 35% ethyl acetate in hexane showed the complete disappearence of starting ester ($R_f = 0.62$) and a new, more polar spot (R_f = 0.22). The reaction mixture was carefully acidified to pH 3.0 by dropwise addition of 2 N HCl and extracted with ethyl acetate. The extract was dried (anhydrous $MgSO_4$), filtered, and concentrated by rotary evaporation. The crude product was purified on a 3 cm diameter column packed with a 15 cm bed of silica gel, using 35% ethyl acetate in hexanes as eluant to afford pure acid **20** (R_f = 0.22, 20 mg, 92%). Analysis of the product by ^{1}H NMR showed epimerization at C-5. The ratio of integral area for the C-5 hydrogen resonance for erythro (δ 2.94) to threo (δ 2.66) diasteromers was 2:1: ^{1}H NMR (**20**, 300 MHz, $CDCl_3$) δ 5.55−5.15 (m, 4H), 4.12−4.00 (m, 1H), 3.95−3.82 (m, 2H), 3.55−3.46 (m, 1H), 2.97−2.90 (m, 1H), 2.70−2.58 (m, 1H) 2.32−2.05 (m, 5H), 2.19 (s, 3H), 2.00−1.93 (q, J = 6 Hz, 2H), 1.70−1.15 (m, 19H), 0.95−0.85 (apparent t, 3H), 0.86 (s, 9H), 0.03−0.00 (m, 6H). The acid **20** was used for the next step without further purification.

Iso[4]-LGE$_2$ (5-Acetyl-6-formyl-9-hydroxy-7(*E*),11(*Z*)-heptadecadienoic Acid). Simultaneous deprotection of acetonide and TBDMS ether and subsequent oxidative cleavage of the vicinal diol[12] was utilized for the synthesis of iso[4]-LGE$_2$. A magnetically stirred solution of acid **20** (18 mg, 0.034 mmol) in acetic acid/water (910 μL, 2/1, v/v) was warmed to 40 °C. After 4 h of stirring, TLC analysis showed that the resulting solution contained completely deprotected triol acid, R_f = 0.14 (20% 2-propanol/1.5% acetic acid in hexanes, v/v/v). This solution was added to sodium metaperiodate (9.2 mg, 0.043 mmol, 1.3 equiv) in acetone−water (4.35 mL, 3/7, v/v). After 1.75 h of stirring at room temperature, the reaction was quenched by addition of ethylene glycol (12.4 mg, 0.20 mmol, 6 equiv). After an additional 15 min at room temperature, the reaction mixture was transferred to a separatory funnel containing diethyl ether (25 mL). The aqueous layer was removed and reextracted with diethyl ether (10 mL). The combined organic extracts were washed with water. The extract was dried (anhydrous $MgSO_4$) and filtered, and *n*-heptane (40 mL) was added to the filtrate. The solvent volume was reduced to about 10 mL by rotary evaporation at 20 °C and another portion of *n*-heptane (20 mL) and diethyl ether (10 mL) was added. The solvents were then removed completely by rotary evaporation using a dry ice−acetone trap. The flask was attached to a vacuum trap cooled to −78 °C, and the *tert*-butyldimethylsilanol byproduct was removed by vacuum transfer into the trap at 0.01 mmHg for 30 min. There was obtained 11 mg (93% material balance) of a clear oil which was free of volatile byproducts and acetic acid. The purity of the diasteromers of iso[4]-LGE$_2$ was determined ^{1}H NMR spectral analysis.[12] The purity of iso[4]-LGE$_2$ was determined by the ratio of integrated area of the aldehydic proton resonance (δ 9.50, d, $J = 4.35$ Hz, 1H) to the terminal methyl proton resonance (δ 0.86, t, 3 H). The integrated aldehydic resonance was 50% of the expected value relative to that of the terminal methyl group. To insure the quantitative integration of the aldehyde resonance, the T_1 relaxation of this resonance was determined by the inversion−recovery technique. Data analysis was performed using Varian Gemini T_1 software program. For iso[4]-LGE$_2$ in $CDCl_3$ at 20 °C the T_1 relaxation of aldehyde signal (δ 9.49, s) is 2.0 s. A pulse sequence delay of at least five times the calculated T_1 for a particular pulse width was used during data acquisition of spectra used for yield determination. A sample of iso[4]-LGE$_2$ (2 mg) was stored in $CDCl_3$ (99.99%, Aldrich, 1 mL ampule) at −78 °C for several weeks, and ^{1}H NMR showed no change in proton resonances. The ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) is consistent with a mixture of iso[4]-LGE$_2$ diastereomers. Characteristic resonances of the major isomers and their assignments include δ 9.50 (d, $J = 4.35$ Hz, 1H, CHO), 4.16 (m, 1H, C-9H), 3.59 (m, 1H, C-6H), 3.00 (m, 1H, C-5H), 2.26 and 2.24 (2s, 3H, $COCH_3$). The spectrum also indicated the presence of other minor isomers which may include a small amount of the α,β-unsaturated aldehyde Δ^6-iso[4]-LGE$_2$ (*vide infra*). Iso[4]-LGE$_2$ was further characterized as the iso[4]-LGM$_2$−TMS-PFB. A solution of iso[4]-LGE$_2$ (~100 μg) in acetonitrile (50 μL) was treated with 3% methoxylamine hydrochloride (300 μL) in water. The mixture was stirred at room temperature for 2 h and then acidified to pH 3 with 1 N HCl. The product was extracted with methylene chloride (1 mL), and solvents were evaporated with a stream of nitrogen. The contents were resuspended in acetonitrile with diisopropylethylamine (20 μL, 10% diisopropylethylamine in acetonitrile), and then pentafluorobenzyl bromide (40 μL, 10% pentafluorobenzyl bromide in acetonitrile) was added. After 10 min at room temperature, volatiles were evaporated with a stream of dry nitrogen. The reaction mixture was resuspended in DMF (7 μL) and bis(trimethylsilyl)trifluoroacetamide (20 μL). The contents were incubated at 37 °C for 10 min, and then volatiles were removed with a stream of dry nitrogen. The HRMS (24 eV) showed the chachteristic ions summarized in Table 2.[4]

Table 2. HRMS of Iso[4]-LGM$_2$−TMS-PFB

formula	ion	*m/z* (calcd)	*m/z* (found)	rel intens
$C_{32}H_{47}F_5N_2O_5Si^+$	M^+	662.3174	662.3162	1.41
$C_{29}H_{41}F_5N_2O_4Si^+$	$M^+ - 72$	590.2725	590.2750	3.52
$C_{24}H_{32}F_5N_2O_5Si^+$	$M^+ - 111$	551.2000	551.2010	16.1
$C_{23}H_{28}F_5N_2O_4Si^+$	$M^+ - 143$	519.1738	519.1720	8.73
$C_{21}H_{22}F_5N_2O_4^+$	$M^+ - 201$	461.1499	461.1476	20.7
$C_{15}H_{15}F_5NO_3^+$	$M^+ - 310$	352.0972	352.0989	9.86
$C_{17}H_{32}NO_2Si^+$	$M^+ - 352$	310.2202	310.2149	0.85
$C_6H_{12}NO^+$	$M^+ - 548$	114.0919	114.1273	100
$C_3H_6NO^+$	$M^+ - 590$	72.0449	72.0562	87.3

Table 3. HRMS of LGM_2−TMS-PFB

formula	ion	*m/z* (calcd)	*m/z* (found)	rel intens
$C_{32}H_{47}F_5N_2O_5Si^+$	M^+	662.3174	662.3174	2.46
$C_{29}H_{41}F_5NO_4Si^+$	$M^+ - 72$	590.2725	590.2651	0.75
$C_{27}H_{36}F_5N_2O_5Si^+$	$M^+ - 71$	591.2313	591.2448	1.54
$C_{26}H_{32}F_5N_2O_4Si^+$	$M^+ - 103$	559.2051	559.2353	8.73
$C_{24}H_{26}F_5N_2O_4^+$	$M^+ - 161$	501.1812	501.1766	2.62
$C_{18}H_{19}F_5NO_3^+$	$M^+ - 270$	392.1285	392.1235	8.60
$C_{14}H_{28}NO_2Si^+$	$M^+ - 392$	270.1889	270.1871	3.94
$C_6H_{12}NO^+$	$M^+ - 548$	114.0919	114.1291	100
$C_3H_6NO^+$	$M^+ - 590$	72.0449	72.0560	81.7

Δ^6-iso[4]-LGE_2 (5-Acetyl-6-formyl-9-hydroxy-6(*E*),11(*Z*)-heptadecadienoic Acid). A solution of iso[4]-LGE_2 (4 mg, 40% aldehydic purity) in $CDCl_3$ (1 mL) was stored at −78 °C. ^{1}H NMR analysis of the product showed the complete disappearance of doublet at δ 9.50 and appearance of a singlet at δ 9.43. The sample was passed through a silica gel plug using ethyl acetate/*n*-heptane/acetic acid (50:48:2, v/v/v). The resulting light yellow product was further purified by HPLC on a Whatman Partisil column (4.6 mm i.d. × 25 cm) employing ethyl acetate/*n*-heptane/acetic acid (40:58:2, v/v/v) as the mobile phase at a flow of 0.8 mL/min. Samples containing 200 μg in 50 μL were injected onto the column. Under these conditions, the retention time of Δ^6-iso[4]-LGE_2 was 16.2 min. The product was collected, and solvents were completely removed under reduced pressure afford Δ^6-iso[4]-LGE_2: ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.43 (s, H, CHO), 6.85 (t, H, J = 7.4 Hz, C-7H), 5.68−5.25 (2H, C-11H, C-12H), 3.80 (m, C-9H), 3.65 (m, C-5H), 2.55−2.345 (2H, C4-H), 2.40−2.20 (4H, C8H, C-2H), 2.04 (s, 3H, acetyl methyl), 2.10−1.90 (2H, C-13H), 1.80−1.10 (10H), 0.87 (t, 3H, J = 6.0 Hz, C-17H); HRMS (24 eV) *m/z* calcd for $C_{20}H_{32}O_5$ (M^+) 352.2249, *m/z* (relative intensity) found 352.2235.

9-(2-Aza-2-methoxyvinyl)-8-(2-aza-1-methyl-2-methoxyvinyl)-12-hydroxyheptadeca-5,10-dienoic Acid (LGM_2). The following synthesis of LGE_2−bismethoxime is the standard procedure for methoximation. A solution of LGE_2 (20 mg, 0.056 mmol) and methoxylamine hydrochloride (60 mg, 0.71 mmol) in anhydrous pyridine (1 mL) was stirred for 24 h. TLC analysis showed the presence of a new spot (R_f = 0.15). The pyridine was evaporated under a stream of nitrogen, and then water (2 mL) was added to the solid residue. The aqueous layer was extracted with ethyl acetate, and the extract was dried ($MgSO_4$) and concentrated. The oily residue was purified by HPLC using a Whatman Partisal column with 50% EtOAC/hexane as eluant at a flow rate of 2 mL/min (retention time 7.8 min) to give LGM_2 (19 mg, 83%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.12 (d, J = 8.24 Hz, 1 H), 5.71−5.49 (m, 2 H), 5.35−5.23 (m, 2 H), 4.22−4.16 (m, 1 H), 3.82 (s, 3 H), 3.72 (s, 3 H), 3.03−2.98 (m, 1 H), 2.46−2.37 (1 H), 2.34-2.28 (m, 2H), 2.15−1.93 (2H), 1.72 (s, 3 H), 1.70−1.51 (4 H), 1.45−1.10 (8 H), 0.88−0.85 (3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.4, 150.6, 150.4, 136.3, 130.2, 127.4, 127.3, 72.2, 61.4, 48.1, 47.9, 45.7, 40.8, 36.8, 31.7, 27.5, 26.3, 25.0, 24.9, 24.4, 22.6, 14.0, 12.0; mass spectrum *m/z* (M^+) calcd for $C_{22}H_{38}O_5N_2$ 410.2781, found 410.2772.

LGM_2 Methyl Ester. Solid KOH (240 mg) was dissolved in water (0.4 mL) in a vial, and diethyl ether (3 mL) was added. The vial was cooled in ice and solid *N*-nitroso-*N*-methylurea (120 mg) was added. On stirring the reaction, a yellow color appeared in the ether layer. The ether layer was carefully pipetted out and added to a solution of LGM_2 (7 mg, 0.017 mmol) in ether (0.5 mL). The reaction mixture was stirred for 5 min. TLC analysis in 50% EtOAc/hexane showed disappearance of starting material (R_f = 0.15) and appearance of two new spots (R_fs = 0.38, 0.42). The solvent was removed *in vacuo*, and the crude product was purified by HPLC using 25% EtOAC/hexane as eluant at a flow rate of 2 mL/min. The major isomer (5.6 mg, 77%) had a retention time of 6.8 min and the minor isomer had a retention time of 7.5 min (1 mg, 14%). **Major isomer:** ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.19 (d, J = 8.14 Hz, 1 H), 5.71−5.53 (m, 2 H), 5.49−5.22 (m, 2 H), 4.12−4.03 (m, 1 H), 3.80 (s, 3 H), 3.79 (s, 3 H),3.76 (s, 3 H), 3.03 (m,1 H), 2.44−2.24 (m, 3 H), 2.04−1.97 (2H), 1.70 (s, 3 H), 1.74−1.19 (13 H), 0.88−0.84 (3 H); mass spectrum *m/z* (M^+) calcd for $C_{23}H_{40}O_5N_2$ 424.2937, found 424.2937. **Minor isomer:** ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.23 (d, J =7.98 Hz, 1 H), 5.62−5.34 (m, 4 H), 4.11−4.01 (m, 1 H), 3.79 (s, 3 H), 3.76 (s, 3 H),3.65 (s, 3 H), 3.01 (m,1 H), 2.38−2.01 (m, 5 H), 1.70 (s, 3 H), 1.70−1.23 (13 H), 0.88−0.85 (3 H); mass spectrum *m/z* (M^+) calcd for $C_{23}H_{40}O_5N_2$ 424.2937, found 424.2935.

LGM_2 PFB Ester. To a solution of LGM_2 (6 mg, 0.015 mmol) and diisopropylamine (10 mg, 0.09 mmol) in acetonitrile (500 μL), was added a solution of pentafluorobenzyl bromide (15 mg, 0.07 mmol) in acetonitrile (100 μL). The reaction mixture was stirred for 6 h at room temperature. TLC analysis in 25% EtOAC/hexane revealed disappearance of starting material (R_f = 0.15) and appearance of two new spots (R_fs = 0.5, 0.43). The solvent was removed *in vacuo*, and the residue was purified by HPLC using 30% EtOAC/hexane as eluant at a flow rate of 2 mL/min. The major isomer (5.3 mg, 65%) had a retention time of 5.8 min and the minor isomer had a retention time of 6.5 min (1.2 mg, 15%). **Major isomer:** ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.08 (d, J = 8.75 Hz, 1 H), 5.51−5.36 (m, 2 H), 5.20−5.13 (m, 2 H), 5.04 (d, J = 1.5 Hz, 2 H), 3.95 (m, 1 H), 3.68, (s, 3 H), 3.66 (s, 3 H), 3.64 (s, 3 H), 2.89 (m,1 H), 2.30−1.99 (m, 3 H), 1.95−1.84 (2 H), 1.62−1.14 (15 H), 0.76−0.73 (3 H); mass spectrum *m/z* (M^+) calcd for $C_{29}H_{39}O_5N_2F_5$ 590.2778, found 590.2930. **Minor isomer:** ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.12 (d, J = 7.95 Hz, 1 H), 5.55−5.16 (m, 2 H), 5.04 (d, J = 1.5 Hz, 2 H), 3.95 (m, 1 H), 3.67 (s, 3 H), 3.66 (s, 3 H), 3.65 (s, 3 H), 2.89 (m,1 H), 2.20−1.87 (5 H), 1.56−1.08 (15 H), 0.76−0.73 (3 H); mass spectrum *m/z* (M^+) calcd for $C_{29}H_{39}O_5N_2F_5$ 590.2778, found 590.2726. A TMS ether was prepared as described above for iso[4]-LGM_2-PFB. The HRMS (24 eV) showed the characteristic ions summarized in Table 3.

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Supporting Information Available: ^{1}H NMR spectra of all new compounds (16 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from ACS; see any current masthead page for ordering information.

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