Total Synthesis of 17-isoLevuglandin E_4 and the Structure of C22-$PGF_{4\alpha}$

Wei Sha and Robert G. Salomon*

Department of Chemistry, Case Western Reserve University, Cleveland, Ohio 44106-7078

rgs@po.cwru.edu

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The isolevuglandin 17-isoLGE_4 (10-acetyl-11-formyl-14-hydroxynonadeca-4(*Z*),7(*Z*),12(E),16(*Z*)-tetraenoic acid) is a levulinaldehyde derivative that is expected to be generated during the free radical-induced oxidation of docosahexaenoic acid. A total synthesis was executed to facilitate detection and identification of 17-isoLGE_4 in biological samples. Conjugate addition of a higher order vinyl cyanocuprate to a γ-alkoxy enone achieved the final carbon−carbon bond formation to complete a convergent elaboration of the 17-isoLGE_4 carbon skeleton. Attempted construction of the requisite vinyl nucleophile synthon using hydrostannylation of an alkyne was foiled by tri-*n*-butylstannyl radical-promoted isomerization of a cis to a trans double bond. Derivatization of 17-isoLGE_4 with methoxylamine under anhydrous or wet conditions generated bismethoximes of 17-isoLGE_4 or the isomerized Δ^{11}-17-isoLGE_4 respectively. Analysis of the mass spectrum of a bismethoxime−pentafluorobenzyl ester−trimethylsilyl ether derivative of 17-isoLGE_4 provided presumptive evidence that an incorrect structure was proposed earlier for C22-$PGF_{4\alpha}$, the only F_4-isoprostane which is produced enzymatically. We conclude that the 22-carbon analogue of $PGF_{2\alpha}$, produced from docosahexaenoic acid by a cyclooxygenase from trout gill, does not have the same side chains as 17-isoLGE_4. Furthermore, we now propose that mass spectral data reported for "C22-$PGF_{4\alpha}$" support a 14-$PGF_{4\alpha}$ structure rather than the 17-$PGF_{4\alpha}$ structure suggested previously.

Introduction

The cyclooxygenase (COX)-promoted oxidation of arachidonic acid (AA) generates the prostaglandin (PG) endoperoxide intermediate PGH_2 that rearranges to PGs[1−5] and levulinaldehyde derivatives, e.g., levuglandin (LG) E_2 (Scheme 1).[6] In vivo, most AA is present as an ester of 2-lyso-phosphatidylcholine (PC). Although AA-PC is not a substrate for COX, free radical-induced oxidation of AA-PC generates phospholipid endoperoxide stereoisomers, isoPGH_2-PC,[7] that rearrange to produce phospholipid esters of LG stereoisomers (referred to collectively as isoLGs). Besides producing mixtures of LG *stereoisomers*, free radical-induced oxidation of AA-PC also results in the production of *structurally isomeric* levulinaldehyde derivatives with nonprostanoid side chains (referred to as iso[*n*]LGs, where *n* is the number of carbons in the carboxyl side chain). LGs react avidly with proteins. Paal-Knorr condensation[8] of isoLGE_2-PC with protein amino groups in conjunction with hydrolytic release of lyso-PC generates the same LGE_2−pyrrole protein adducts as are formed from LGE_2 (Scheme 1). IsoLGE_2-derived and iso[4]LGE_2-derived protein adducts are produced during free radical-induced oxidation of low-density lipoprotein (LDL).[9,10] Such LG-modified proteins are present in human blood, and mean levels in patients with atherosclerosis are nearly double those in controls.[11]

Docosahexaenoic acid (DHA) is a richly polyunsaturated fatty acid that is abundant in human brain cortex and retina phospholipids, as well as in fish oil. In analogy with AA-PC, free radical-induced oxidation of DHA-PC can produce PG-like compounds (Scheme 2). These 22-carbon PG analogues were named neuroprostanes (NPs) because DHA esterified in lipids is abundant in brain.[12] Because they have four C=C bonds, the NP analogues of $PGF_{2\alpha}$ have also been called F_4-isoprostanes.[13] Levels of F_4-NPs are significantly higher in cerebrospinal fluid from Alzheimer's disease patients than from controls.[12] Esterified F_4-NPs, presumably in phospholipids, were isolated from brain.[12] Because free-radical-induced oxidation is not regioselective, eight structurally isomeric families (series) of F_4-NP (isoPGF_4) stereoisomers are produced from DHA.[12,13] Each series is distinguished with a prefix that refers to the position of the allylic hydroxyl

(1) Hamberg, M.; Samulesson, B. *Proc. Natl. Acad. Sci. U.S.A.* **1973**, *70*, 899−903.
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(9) Salomon, R. G.; Subbanagounder, G.; Singh, U.; O'Neil, J.; Hoff, H. F. *Chem. Res. Toxicol.* **1997**, *10*, 750−759.
(10) Salomon, R. G.; Sha, W.; Brame, C.; Kaur, K.; Subbanagounder, G.; O'Neil, J.; Hoff, H. F.; Roberts, L. J., II *J. Biol. Chem.* **1999**, *274*, 20271−20280.
(11) 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*, 536−545.
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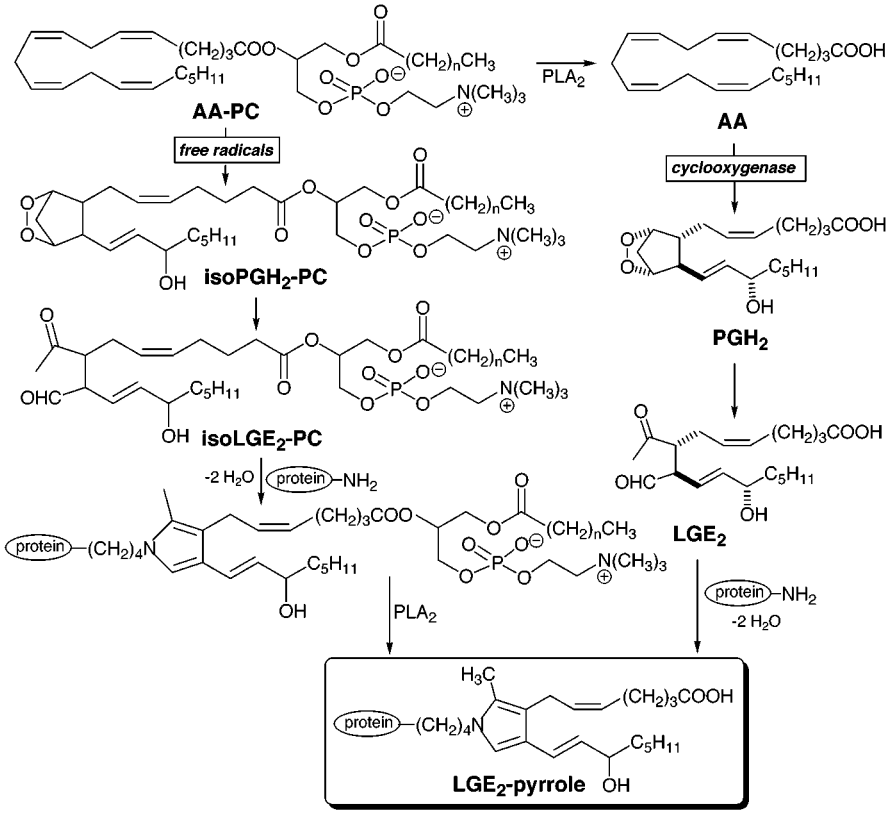
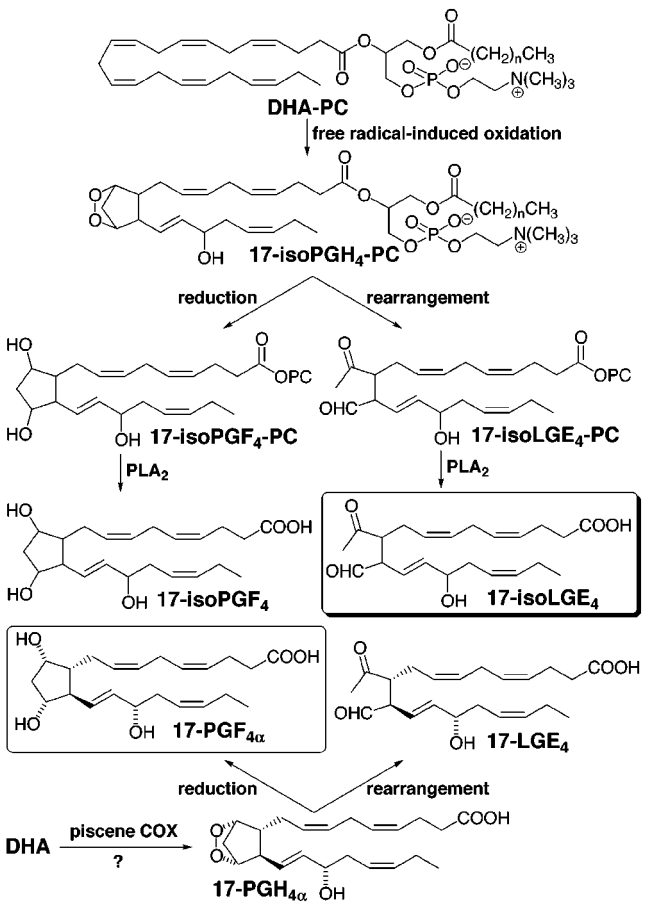
Scheme 1



Scheme 2



substituent. Free radical-induced oxidation of DHA phospholipids, e.g., DHA-PC, is presumed to generate endoperoxide intermediates, e.g., 17-isoPGH$_4$-PC that delivers 17-isoPGF$_4$-PC by reduction. Because rearrangements of the bicyclic PG endoperoxide nucleus[6] and of PGH$_2$ itself occur readily in aqueous solution at 37 °C ($t_{1/2}$ = 5 min), rearrangement of 17-isoPGH$_4$-PC is expected to produce the 22-carbon isoLGE$_2$-PC analogue 17-isoLGE$_4$-PC (see Scheme 2).

DHA *is not* a substrate for mammalian COX, and it inhibits cyclooxygenation of AA.[14−18] Apparently, this selectivity evolved as an adaptation to a nonaquatic environment because a piscene COX enzyme (from rainbow trout) does not discriminate against DHA. In fact, DHA *is* an excellent substrate for the piscine COX. Incubation of DHA with trout gill homogenate generates a 22-carbon analogue of PGF$_{2\alpha}$, that was called C22-PGF$_{4\alpha}$, for which a 17-PGF$_{4\alpha}$ structure (see Scheme 2) was proposed based on mass spectral studies.[19] Presumably, this piscene cyclooxygenation involves an endoperoxide intermediate, 17-PGH$_{4\alpha}$, which could also afford 17-LGE$_4$ by rearrangement.

We now report a total synthesis of 17-isoLGE$_4$ that provides an authentic standard and derivatives that will be useful for characterizing the products of DHA oxidation, as well as exploring their protein adduction chemistry and biological activities. Mass spectral studies on

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(15) Khair-el-Din, T. A.; Sicher, S. C.; Vazquez, M. A.; Wright, W. J.; Lu, C. Y. *Immunol.* **1995**, *154*, 1206−1296.
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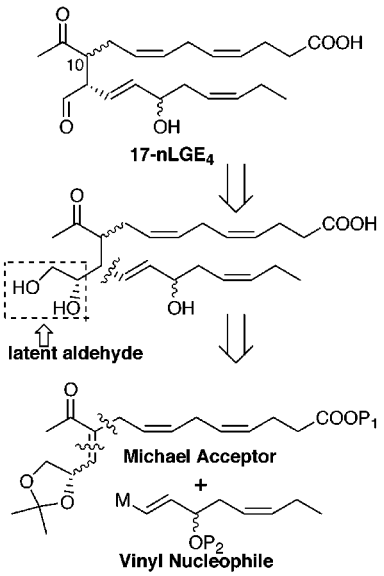
(18) Rao, G. H.; Radha, E.; White, J. G. *Biochem. Biophys. Res. Commun.* **1983**, *117*, 549−555.
(19) Mai, J.; Goswami, S. K.; Bruckner, G.; Kinsella, J. E. *Prostaglandins* **1981**, *21*, 691−698.

Scheme 3



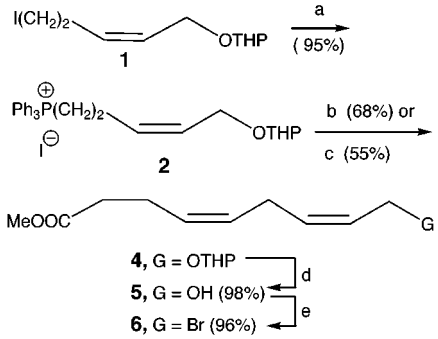
derivatives of 17-isoLGE$_4$ suggest that C22-PGF$_{4\alpha}$ is a 14-PGF$_{4\alpha}$ and not a 17-PGF$_{4\alpha}$ as proposed previously.

Results and Discussion

The Synthetic Design. The presence of a sensitive vinylogous aldol array, with its proclivity toward dehydration and allylic rearrangement, recommended the use of a vicinal diol as a latent aldehyde (Scheme 3) as we had done previously for total syntheses of LGE$_2$ and iso-[4]LGE$_2$.[20,21] The proclivity of its labile doubly allylic methylene toward hydrogen atom abstraction provided an additional challenge for handling 17-isoLGE$_4$ and precursors. Conjugate addition of a vinyl nucleophile to a γ-alkoxy enone Michael acceptor was planned as the key carbon−carbon bond forming step that would complete the requisite carbon skeleton. Two of the three stereocenters (C10 and C11 according to numbering as a nonatetraenoic acid derivative) in 17-isoLGE$_4$ will be destroyed upon condensation with ϵ-amino groups of protein lysyl residues to generate pyrrole adducts. Furthermore, free radical-induced oxidation is expected to generate LGE$_4$s as stereoisomeric mixtures. Therefore, no effort was made to achieve stereocontrol.

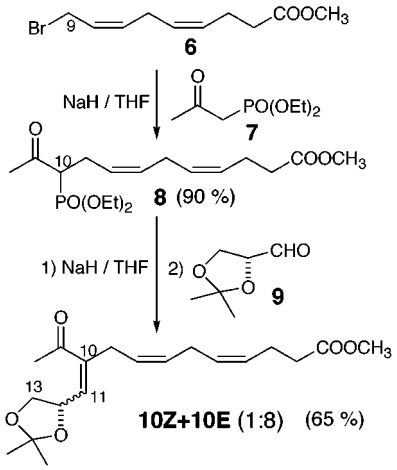
Synthesis of Michael Acceptor 10. The Michael acceptor **10** for the 17-isoLGE$_4$ synthesis possesses two homoallylic cis double bonds in the side chain. There are two common methods to construct homoallylic cis double bonds. One is to assemble a homoallylic diyne and then do a controlled double hydrogenation.[22] The other is to construct a cis double bond by a Wittig reaction. The Wittig method was chosen to avoid possible purification complications. Phosphonium iodide salt **2** was prepared from readily available iodide **1** (Scheme 4). It is worth mentioning that the THP ether was a suitably stable protecting group while initial attempts to employ an α-ethoxyethoxy ether failed because this protecting group did not survive in the subsequent reactions. Coupling of

Scheme 4[a]



[a] Key: (a) Ph_3P, CH_3CN, 38 °C, 48 h; (b) *n*-BuLi, HMPA, $CH_3O_2C(CH_2)_2CHO$ (**3**), THF, −78 to 0 °C; (c) $[(CH_3)_3Si]_2NLi$, HMPA, THF, −78 to 0 °C; (d) PPTS, CH_3OH, 38 °C, 11 h; (e) $Ph_2PCH_2CH_2PPh_2$, CBr_4, CH_2Cl_2, 0 °C to rt.

Scheme 5



phosphonium salt **2** with aldehyde **3** (Scheme 4) was carried out by the methodology of E. J. Corey[23−26] as well as by a newer alternative method.[22,27,28] Both methods gave similar yields of diene **4**. Alkylation of an excess of diethylphosphonoacetone (**7**) with the chemically sensitive bromide **6** afforded ketophosphonate **8** in good yield and with little dialkylation. Horner−Emmons condensation of the sodium salt of **8** with isopropylidene-D-glyceraldehyde (**9**) provided chiral nonracemic enones **10E** and **10Z** (8:1) in 65% yield (Scheme 5). It is interesting to notice that a higher *E*/*Z* ratio of enone was observed than in the similar LGE$_2$ synthesis,[20] probably owing to the longer and more rigid side chain. Geometrical assignments for these isomers were made by ^{1}H NMR after isolation by HPLC. The ^{1}H NMR spectrum of the major isomer **10E** exhibits a downfield olefinic resonance at δ 6.54 (d, 1H, J = 8.2 Hz) which is assigned to the C-11 vinyl methine proton. The ^{1}H NMR spectrum of the minor isomer **10Z** exhibits an upfield shift of 0.76

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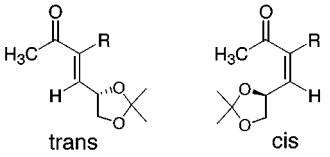
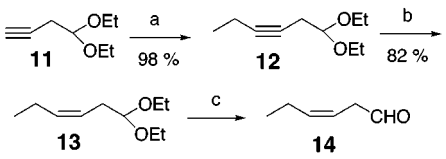
Table 1. ^{1}H NMR Chemical Shift Comparisons of the H_β Resonances for Cis and Trans α,β-Unsaturated Carbonyl Compounds



R	H_β trans	H_β cis	Δ_{t-c}	ref
$-CH{=}CH_2$	6.59	6.00	0.59	20
$-CH_2CH{=}CH-(CH_2)_3CO_2CH_3$	6.49	5.72	0.77	20
$-(CH_2)_3CO_2CH_3$	6.50	5.72	0.78	21
$-CH_2CH{=}CH-C_5H_{11}$	6.48	5.74	0.74	30
$-CH_2CH{=}CH-CH_2CH{=}CH-(CH_2)_2CO_2CH_3$	6.54	5.78	0.76	this study

Scheme 6[a]

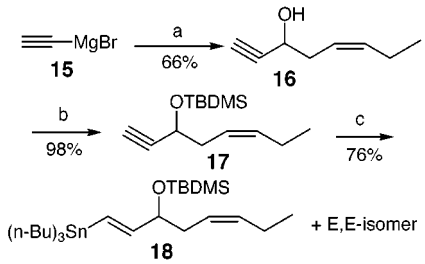


[a] Key: (a) *n*-BuLi, HMPA, CH_3CH_2I, −78 to 0 °C; (b) *i*-BuMgBr, $TiCpCl_2$, ether, 0 °C to rt; (c) AcOH−H_2O (4:1), DBHQ, rt, 1 h.

ppm for this C-11 vinyl methine proton resonance, which occurs at δ 5.78. Upfield chemical shifts of similar magnitude for structurally similar *Z* versus *E* enones were reported previously.[20,21,29,30] Similar variations in chemical shifts of H_β of cis and trans α,β-unsaturated carbonyl compounds are well documented (Table 1).[31] The ratio of *E* to *Z* isomers was calculated by dividing the integrated area of the C-11 vinyl methine resonance (δ 6.54 ppm) of the *E* isomer by the integrated area of the C-11 vinyl methine resonance (δ 5.78 ppm) of the *Z* isomer. The mixture of isomeric enones, **10E** and **10Z**, was used without separation for the next step.

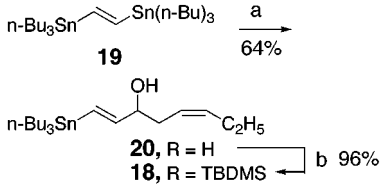
Synthesis of Vinylstannane 18. The racemic lower side chain fragment for 17-isoLGE$_4$ was assembled by addition of a two-carbon nucleophile to (*Z*)-3-hexenal (**14**). This volatile and sensitive β,γ-unsaturated aldehyde has a propensity to isomerize to the more stable (*E*)-2-hexenal. Therefore, solvents were removed from crude (*Z*)-3-hexenal (**14**) under pressure greater than 80 mmHg, and **14** was used immediately for the next step without extensive purification (Scheme 6). Attempted preparation of vinylstannane **18** by free radical-induced addition of tributyltin hydride to alkyne **17** (Scheme 7) delivered mixtures of the *E*,*Z* diene **18** and its *E*,*E* stereoisomer. The undesired cis−trans isomerization presumably arises from the reversible addition of a tributylstannyl radical to the cis C=C bond. Therefore, addition of *trans*-2-(tri-*n*-butylstannyl)vinyllithium to freshly prepared (*Z*)-3-hexenal (**14**) was employed to produce **18**[21] (Scheme 8). *trans*-2-(Tri-*n*-butylstannyl)ethylene (**19**) is readily available in two steps from acetylene.[32] Transmetalation of **19** in THF to 1 equivalent of *n*-BuLi provides 2-(tri-*n*-butylstannyl)vinyllithium. Addition of 1 equivalent of (*Z*)-3-hexenal (**14**) to 1.5 equivalents of this stannylvinyllithium delivered the stannylvinyl alcohol **20** in 64% yield.

Scheme 7[a]



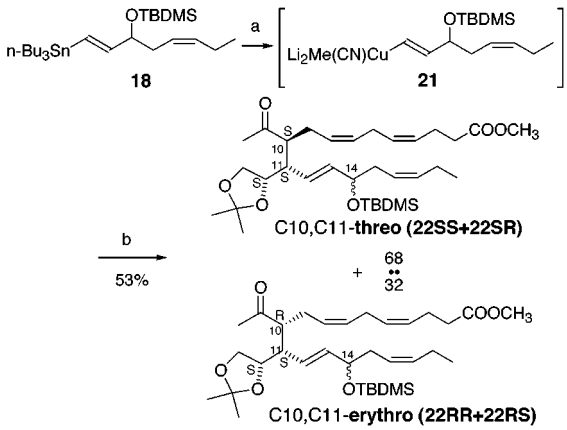
[a] Key: (a) **14**, THF, 0 °C to rt; (b) TBDMS-Cl, imidazole, DMF, 20 h, rt; (c) Bu_3SnH, AIBN, 80 °C, 2 h.

Scheme 8[a]



[a] Key: (a) *n*-BuLi, **14**, THF, −78 to 0 °C; (b) TBDMS-Cl, imidazole, DMF, 15 h, rt.

Scheme 9[a]



[a] Key: (a) CuCN, MeLi, THF, then **18**, rt, 1.5 h; (b) **10Z**+**10E**, THF, −78 °C for 30 min, then −30 °C for 30 min.

Synthesis of 17-isoLGE$_4$. Previous syntheses of PGs[33] and LGs,[20,21,29,30] have demonstrated the utility of vinylstannanes as precursors of a lower side chain vinyl nucleophile. Accordingly, these intermediates were applied to the synthesis of 17-isoLGE$_4$ precursor **22**. A convergent construction of the 17-isoLGE$_4$ carbon skeleton was accomplished as outlined in Scheme 9. The mixed higher order cyanocuprate **21** was prepared by transmetalation of vinylstannane **18** with $Li_2Me_2Cu(CN)$.[33] Addition of the mixture of isomeric enones **10E** and **10Z** (8:1) to vinylcyanocuprate **21** provided a mixture of diastereomeric conjugate addition products **22**. The diastereomers with 10,11-erythro and threo side-chain arrangements were separated by flash column chroma-

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(30) Roy, S. C.; Nagarajan, L.; Salomon, R. G. *J. Org. Chem.* **1999**, *64*, 4, 1218−1224.
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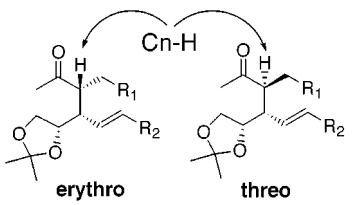
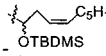
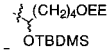
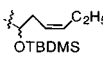
Table 2. ^{1}H NMR Chemical Shift Comparisons of the Cn-H Resonances for a Series of Erythro and Threo Conjugate Addition Products



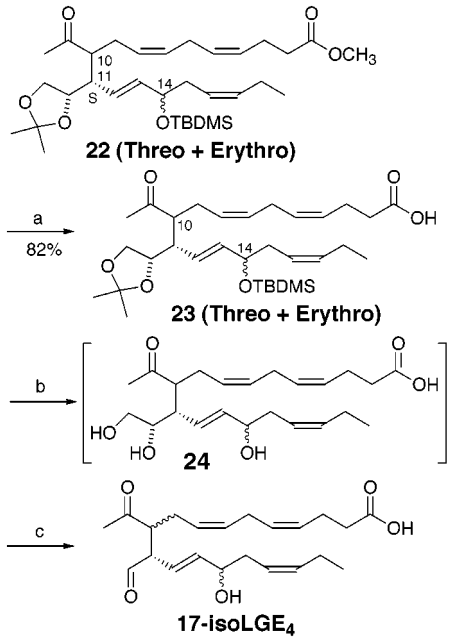
R_1	R_2	Cn-H erythro	Cn-H threo	Cn-H Δ_{e-t}	C(n+1)-H threo	ref.
-CH=CH_2	H	3.10	2.84	0.26	2.58	20
-CH=CH_2		3.06	2.71	0.35	2.49	20
-CH=CH-$(CH_2)_3$COOMe		2.99	2.58	0.41	2.54	20
-$(CH_2)_2$COOEt	H	2.97	2.60	0.37	2.47	21
-$(CH_2)_2$COOEt		2.94	2.64	0.30	2.44	21
-CH=$CH_2C_5H_{11}$		3.04	2.66	0.34	2.49	30
-CH=CH_2CH_2-CH=$CHCH_2$-CH_2COOCH$_3$		3.05	2.74	0.33	2.55	this study

tography to provide C10,C11-threo (**22SS**+**22SR**) and C10,C11-erthyro (**22RR**+**22RS**) isomers in a 68:32 ratio. The absolute configurations of the C-10 and C-14 stereocenters in the conjugate addition products are specified by appropriate configurational designations (*R* or *S*). The overall yield of conjugate addition products was 53% based on the enone reacted.

Two asymmetric centers are created by the conjugate addition reaction and eight diastereomers of **22** are possible since the lower side chain is racemic. However, conjugate addition of vinyl nucleophiles to (*S*)γ-alkoxy enones is a stereoselective reaction.[20,29] The alkoxy α to the carbonyl in glyceraldehyde acetonide serves as a chiral auxiliary directing enantioselective creation of the C-11 stereocenter. Thus, only two diastereomeric pairs of epimeric products were obtained because the *S* configuration was generated enantioselectively at C-11. The stereochemical assignments of the conjugate addition products **22SS**, **22SR** (threo) and **22RS**, **22RR** (erythro) are based on the similarity of the C-10 proton resonance chemical shifts with those reported previously for analogous LGE_2, iso[4]LGE_2, and iso[7]LGD_2 precursors (Table 2).[20,21,29,30] A distinguishing characteristic of the ^{1}H NMR spectra of the erythro and threo conjugate addition products is the chemical shift of the methine hydrogen resonance α t*o* the ketone carbonyl. For the C10,C11-erythro derivatives **22RR** and **22RS**, this resonance occurs at δ 3.05 ppm. For the C10,C11-threo derivatives **22SS** and **22SR**, this resonance is shifted upfield to δ 2.74 ppm.

Saponification of Methyl Ester 22. Initial attempts at saponification of the diastereomers **22** with bases in *homogeneous* solutions was either too harsh leading to decomposition (NaOH,[20] LiOH[34]) or too mild leading to little conversion ($Ba(OH)_2$,[35] K_2CO_3[36]). Saponification was finally carried out successfully in a *heterogeneous* LiOH/H_2O/THF mixture[27] (Scheme 10). It should be noted that saponification of either pure C10,C14-threo or C10,C14-erythro isomer of **22** or mixtures of C10,C14-threo and C10,C14-erythro **22** isomers (68/32) all lead to the same mixture of diastereomeric carboxylic acids **23** owing to epimerization at the C-10 center during ester hydrolysis.[20] The chemical shift position of the C10 proton resonance for the C10,C14-erythro isomers occurs at δ 3.06 ppm while that for the C10,C14-threo isomers occurs at δ 2.75 ppm. The ratio of C10,C14-erythro to C10,C14-threo isomers of **23** was 30:70 based on ^{1}H NMR analysis.

Scheme 10[a]



[a] Key: (a) LiOH, THF−H_2O (5:1), rt, 13 h; (b) AcOH−H_2O (2:1), 40 °C, 4 h; (c) $NaIO_4$, acetone−H_2O (30%), rt, 1.75 h.

In comparison to the corresponding intermediates in the previous LGE_2 synthesis, precursors **22** and **23** of 17-nLGE_4 are much more chemically sensitive. Special precautions were taken, because the presence of a 1,4-diene array makes these compounds prone to free radical oxidation in air. To ensure the absence of trace peroxides, all solvents were distilled and purged with argon before use. The antioxidant butylated hydroxytoluene (BHT) or the free radical scavenger 4-hydroxy-TEMPO were added to the reaction mixtures and products or their solutions for storage.

Consecutive Hydrolysis and Oxidative Cleavage of 23. Generation of 17-isoLGE$_4$. Simultaneous deprotection of the acetonide and TBDMS ether by treatment with acetic acid−water (2:1 v/v) at 40 °C for 4 h, followed by oxidative cleavage of the intermediate vicinal diol **24** with sodium periodate afforded a mixture of 17-isoLGE$_4$ diastereomers (Scheme 10). Excess periodate was quenched by adding an excess of ethylene glycol. The ^{1}H NMR spectrum of 17-isoLGE$_4$ shows a doublet at δ 9.48 (J = 6.0 Hz) for the aldehyde hydrogen. This is similar to the corresponding resonance at δ 9.46 (J = 6.1 Hz) in the spectrum of LGE_2,[20] and at δ 9.50 (J = 4.4 Hz) in the spectrum of iso[4]LGE_2 [21] (Figure 1). The three methine resonances at δ 3.02, 3.58, and 4.20 in the spectrum of 17-nLGE_4 closely match the corresponding resonances a δ 2.94, 3.50, and 4.32 in the spectrum of LGE_2[20] and δ 3.00, 3.56, and 4.16 in the spectrum of iso[4]LGE_2.[21] It is

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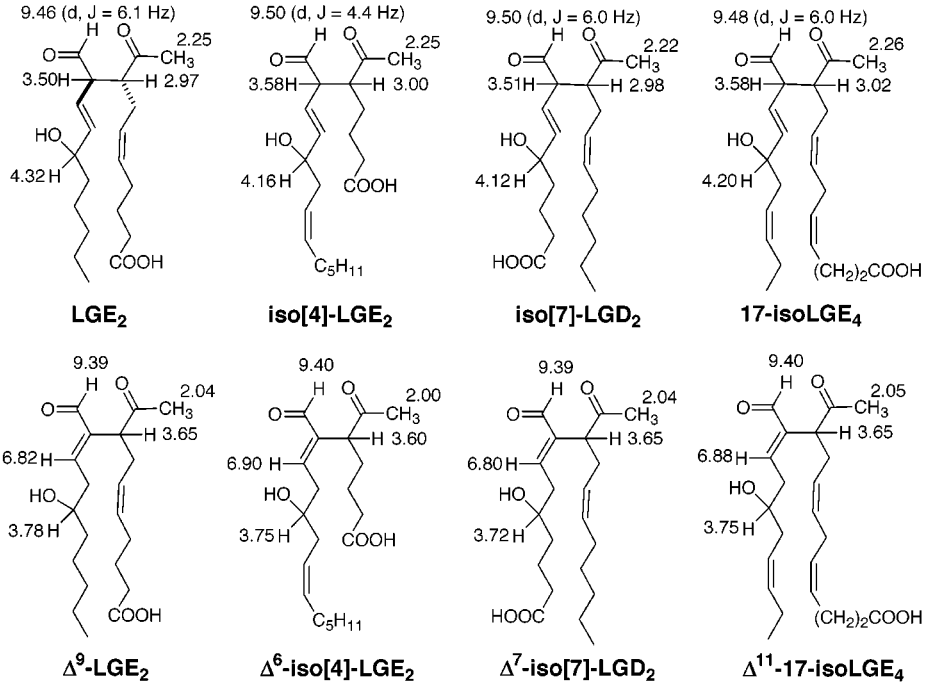
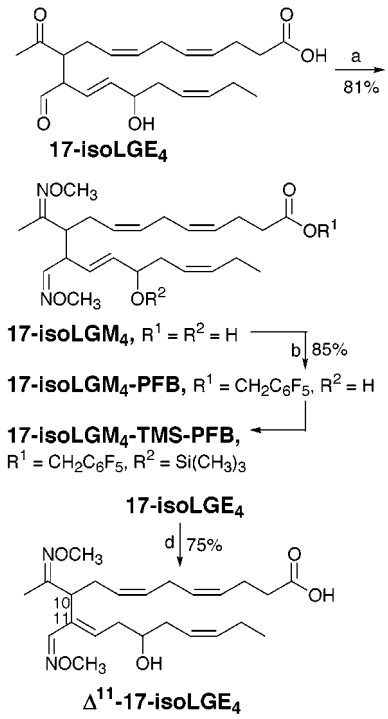


Figure 1. H NMR comparison of various levuglandins and isolevuglandins and their C−C double bond shifted isomers.

noteworthy that, because the lower side of 17-isoLGE$_4$ is very similar to that of iso[4]LGE$_2$, the C11−H, C14−H, and even CHO proton resonances are very close to the corresponding resonances for iso[4]LGE$_2$. Hydrogen NMR spectra of LGE$_2$, various isoLGs and 17-isoLGE$_4$ along with their conjugated isomers are compared in Figure 1. The purity of 17-isoLGE$_4$ was calculated on the basis of ^{1}H NMR analysis based on the ratio of integrated area of the aldehydic hydrogen resonance (δ 9.50, d, J = 4.0 Hz, 1H) to the resonance for the hydrogen at C14 (δ 4.10−4.22, m, 1H).[30] The integrated aldehydic resonance was 30−50% of the expected area relative to that of the C14 hydrogen. 17-isoLGE$_4$ was characterized further as more stable bis methoxime derivatives.

Derivatization of 17-isoLGE$_4$. As for LGE$_2$ and other isoLGs, 17-isoLGE$_4$ is a chemically sensitive vinylogous β-hydroxy aldehyde. Isolation and purification of 17-isoLGE$_4$ is complicated by a proclivity toward dehydration as well as isomerization from the β,γ-unsaturated aldehyde into the conjugated α,β-unsaturated isomer. However, methoxime derivatives of LGs and isoLGs are more stable. They even survive GC analysis, and pentafluorobenzyl (PFB) esters of methoxime trimethylsilyl (TMS) ethers of LGs and isoLGs have been exploited for GC-MS analysis.[21,30] Reaction of 17-isoLGE$_4$ (Scheme 11) with methoxylamine hydrochloride in anhydrous pyridine gave the bismethoxime 17-isoLGM$_4$ that was purified by flash chromatography and HPLC. 17-isoLGM$_4$ is a mixture of syn and anti methoxime stereoisomers which were inseparable chromatographically. A pentafluorobenzyl (PFB) ester of 17-isoLGM$_4$ was prepared by treatment with pentafluorobenzyl bromide and diisopropylamine. For mass spectral analysis, the hydroxyl group in 17-isoLGM$_4$-PFB was silylated by treatment with *N*,*N*′-bis-(trimethylsilyl)-trifluoroacetamide to provide the more volatile 17-isoLGM$_4$-TMS-PFB (vide infra). Methoximation could also be achieved in aqueous solution, so it is an especially convenient reaction for derivatization of biological samples. However, methoximation of 17-isoLGE$_4$ in aqueous solution generated mainly Δ^{11}-17-isoLGM$_4$ instead of 17-isoLGM$_4$ (Figure 2). Thus, isomerization of 17-isoLGE$_4$ from a β,γ-unsaturated aldehyde into a conjugated α,β-unsaturated isomer accompanied methoximation in aqueous solution.

Scheme 11[a]



[a]Key: (a) MeONH$_3$Cl, py (anhydrous), rt, 15 h; (b) C$_6$F$_5$CH$_2$Br, *i*-Pr$_2$NH, MeCN, rt, 8 h; (c) BTMSTFA, DMF, 25 min, 37 °C; (d) MeONH$_3$Cl, py (wet), rt, 24 h.

Mass Spectral Characterization of 17-isoLGM$_4$. 17-isoLGM$_4$-TMS-PFB was characterized by electron impact (EI, 24 eV) high-resolution mass spectrometry (HRMS). Characteristic mass spectral fragments of 17-isoLGM$_4$-TMS-PFB are summarized in Figure 3. The

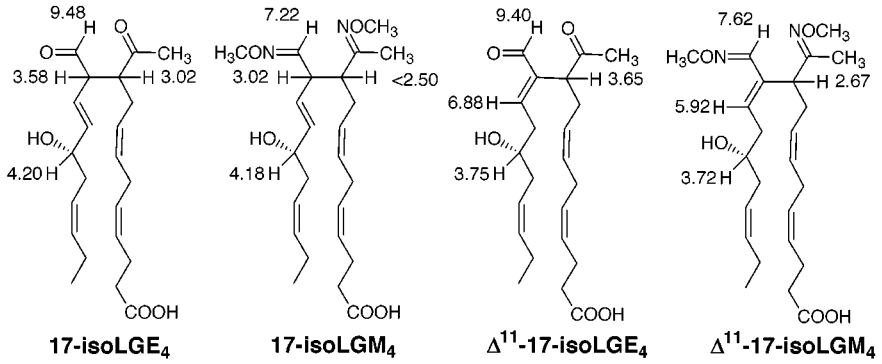


Figure 2. ^{1}H NMR comparison of bismethoxime derivatives of isolevuglandin E_4 and its double bond shifted isomer.

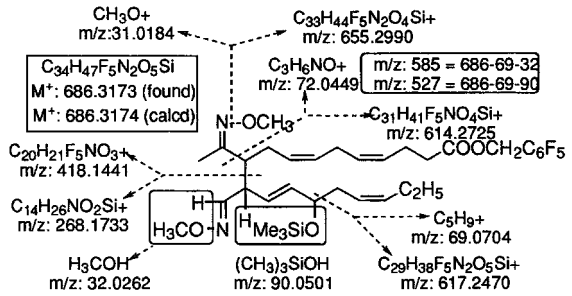


Figure 3. Charachteristic Ions from 17-isoLGE$_4$-TMS-PFB.

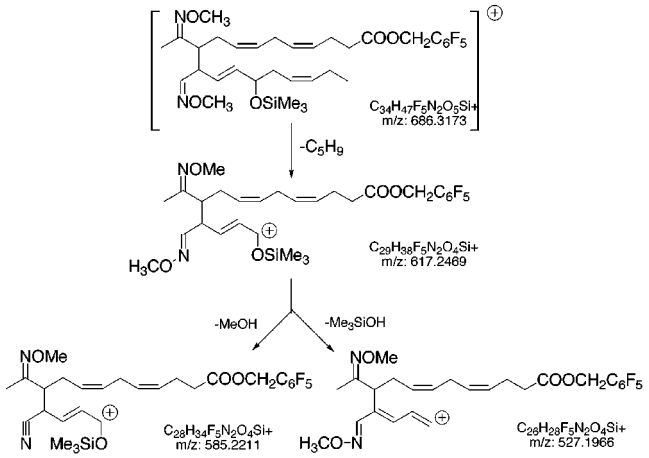
Table 3. HRMS (24 eV) of 17-isoLGM$_4$-TMS-PFB

formula	ion	*m/z* (calcd)	*m/z* (found)	rel Intensity
$C_{34}H_{47}F_5N_2O_5Si^+$	M^+	686.3174	686.3173	12.07
$C_{33}H_{44}F_5N_2O_4Si^+$	$M^+ - 31$	655.2990	655.2976	4.31
$C_{29}H_{38}F_5N_2O_5Si^+$	$M^+ - 69$	617.2470	617.2469	100.00
$C_{31}H_{41}F_5NO_4Si^+$	$M^+ - 72$	614.2725	614.2721	1.05
$C_{28}H_{34}F_5N_2O_4Si^+$	$M^+ - 101$	585.2208	585.2211	15.52
$C_{26}H_{28}F_5N_2O_4^+$	$M^+ - 159$	527.1969	527.1966	16.55
$C_{20}H_{21}F_5NO_3^+$	$M^+ - 268$	418.1441	418.1442	10.12
$C_{14}H_{26}NO_2Si^+$	$M^+ - 418$	268.1733	268.1730	1.42
$C_3H_6NO^+$	$M^+ - 614$	72.0449	72.0453	2.38

calculated and experimentally found *m/z* values and relative intensities for the ions from 17-isoLGM$_4$-TMS-PFB are summarized in Table 3. Several peaks are diagnostic for LGs and isoLGs as a family but do not distinguish between different members of the family. Thus, like LGM$_2$, iso[4]LGM$_2$ and iso[7]LGM$_2$-TMS-PFBs, 17-isoLGM$_4$-TMS-PFB showed a $C_3H_6NO^+$ ion peak at *m/z* 72 which corresponds to the methoxime derivative of an acylium ion (*m/z* 43) as well as an ion at *m/z* 614, corresponding to loss of the methoxime of an acyl group.

Especially characteristic of 17-isoLGM$_4$-TMS-PFB are ions at *m/z* 268 and 418 corresponding to the fragmentation of the molecule between the acetyl- and formyl-substituted tertiary carbons (the lower and upper halves of the molecule). The corresponding derivatives in iso[4]-LGE$_2$, iso[7]LGD$_2$, and LGE$_2$ showed similar fragmentations resulting in ions at *m/z* 310/352, *m/z* 196/466, and *m/z* 270/392, respectively. Another characteristic fragmentation of LG and isoLG bismethoxime pentafluorobenzyl ester trimethylsilyl ether derivatives occurs between the silyloxy-substituted allylic carbon and the neighboring methylene group. Analogous to this fragmentation of iso[4]LGM$_2$, iso[7]LGM$_2$, and LGM$_2$-TMS-PFB, i.e., *m/z* 111/551, *m/z* 267/395, and *m/z* 71/591, loss of carbons 15−19 produced the abundant base ion *m/z* 617. This major ion can undergo further fragmentations

Scheme 12

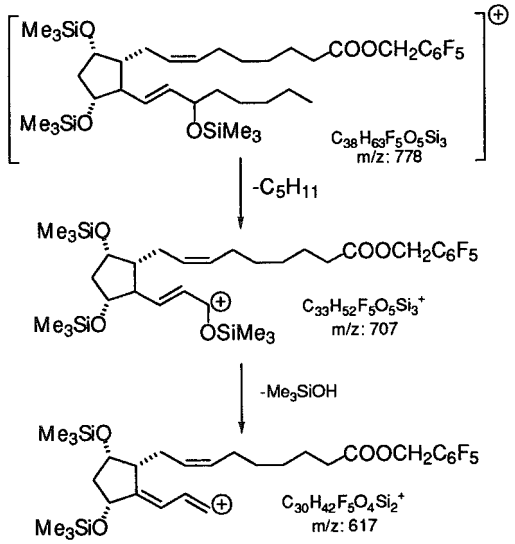


(Scheme 12), i.e., loss of trimethylsilanol to produce an ion of *m/z* 527 and loss of methanol to produce an ion of *m/z* 585. Thus GC-MS analysis of 17-isoLGM$_4$-TMS-PFB further confirmed the structure of 17-isoLGE$_4$. This derivative can be further used as an authentic standard to detect and confirm the formation of 17-isoLGE$_4$ in the in vitro free radical oxidation of docosahexaenoic acid (DHA).

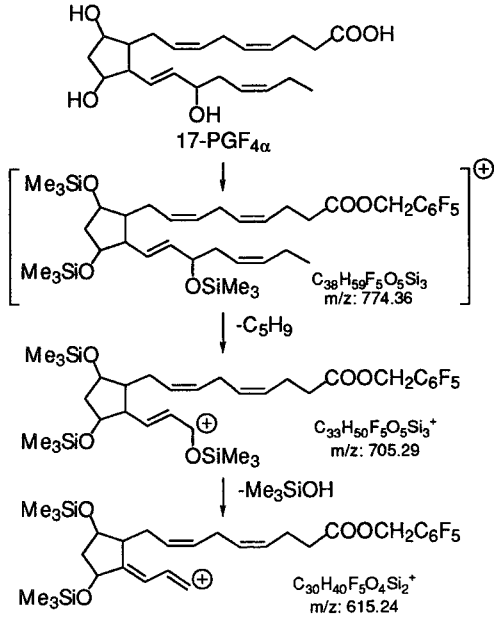
A Revised Structure for the Piscene C22-PGF$_{4\alpha}$. Mass spectral fragmentations that are characteristic of the lower side chain in 17-isoLGM$_4$-TMS-PFB (shown in Scheme 12) include $M^+ - C_5H_9$ which produces *m/z* 617 and $M^+ - C_5H_9$-Me$_3$SiOH which generates *m/z* 527. Analogous fragmentations are also found in the mass spectrum reported previously for bishomoPGF$_{2\alpha}$-trisTMS-PFB.[19] Thus, a prominent peak at *m/z* 707 corresponds to loss of C_5H_{11} and an especially intense peak at *m/z* 617 corresponds to $M^+ - C_5H_{11} -$ Me$_3$SiOH (Scheme 13). If C22-PGF$_{4\alpha}$, a product of a piscene COX oxygenation of DHA, is a 17-PGF$_{4\alpha}$, fragmentations analogous to those observed for 17-isoLGM$_4$-TMS-PFB should produce prominent peaks at *m/z* 705 ($M^+ - C_5H_9$) and *m/z* 615 ($M^+ - C_5H_9 -$ Me$_3$SiOH) in the mass spectrum of C22-PGF$_{4\alpha}$-trisTMS-PFB (Scheme 14). In fact, no peaks are present at these *m/z* in the mass spectrum of the C22-PGF$_{4\alpha}$-trisTMS-PFB. The structure suggested previously[19] is apparently incorrect.

Knowing the correct structure of C22-PGF$_{4\alpha}$ is important because it is the only F_4-isoprostane produced enzymatically. It seems reasonable to presume that interaction of the AA or DHA carboxyl group is important for substrate binding by COX enzymes. Therefore, based on the presumption that the mammalian and piscene

Scheme 13

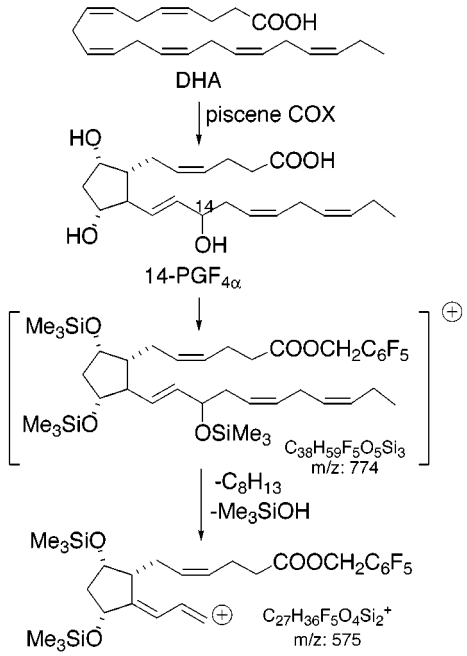


Scheme 14



COXs have similar structures, we postulated that the upper (carboxylic) side chain of C22-PGF$_{4\alpha}$ should resemble the upper 7-carbon side chain of PGF$_{2\alpha}$ as closely as possible. This is best accommodated if the C22-PGF$_{4\alpha}$ is a 14-PGF$_{4\alpha}$, with a 6-carbon carboxylic side chain, rather than a 17-PGF$_{4\alpha}$ with a 9-carbon carboxylic side chain. The mass spectrum of the C22-PGF$_{4\alpha}$-trisTMS-PFB[19] supports a 14-PGF$_{4\alpha}$ structure. Thus, a prominent peak at *m*/*z* 575 corresponds to $M^+ - C_8H_{13} - Me_3SiOH$. Therefore, we now suggest that the 22-carbon analogue of PGF$_{2\alpha}$ produced by the action of piscene COX upon DHA is 14-PGF$_{4\alpha}$ (Scheme 15).

Scheme 15



Experimental Procedures

General Methods. ^{1}H and ^{13}C NMR spectra were recorded at 300 and 75 MHz, respectively, and are reported as described previously.[20,21,29,30] High-resolution mass spectra, solvent purification, and chromatography were performed as usual.[20] All reactions conducted in an inert atmosphere were in argon unless otherwise specified. 2(*Z*)-1-(2-Tetrahydropyranyloxy)-5-iodopentene (**1**),[37] isopropylidene-D-glyceraldehyde (**9**),[38,39] 1,1-diethoxybut-3-yne (**11**),[40] and *trans*-bis(tri-*n*-butylstannyl)-ethylene (**19**)[32] were prepared by literature procedures. Methyl 4-oxobutanoate (**3**) was also prepared by a literature procedure[41] except that pyridinium chlorochromate (1.4 equiv) was used as oxidant instead of pyridinium dichromate.

5-(2-Tetrahydropyranyloxy)-3(*Z*)-pentenyltriphenylphosphonium Iodide (2). Pentenyl iodide (**1**, 2.4 g, 8.11 mmol), Ph_3P (6.38 g, 24.32 mmol, 3.0 equiv), and a few milligrams of $CaCO_3$ in anhydrous acetonitrile (25 mL) were stirred at 38 °C for 48 h (monitoring by TLC until the total disappearance of the iodide).[26] The reaction mixture was then filtered through a Celite pad, and the filtrate was rotary evaporated. The resulting yellow solid was washed with diethyl ether (5 × 50 mL) and recrystallized from THF, and the solvent was evaporated thoroughly into a dry ice−acetone cooled trap at 0.5 mmHg to deliver the phosphonium iodide **2** (4.35 g, 95% yield) as light yellow prisms: mp 255−257 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.86−7.79 (9H), 7.74−7.67 (6H), 5.88 (dd, 1H, J = 6.7, 4.0 Hz), 5.60 (apparent q, 1H, J = 6.7 Hz), 4.50 (s, 1H), 4.04 (dd, 1H, J = 6.6, 6.2 Hz), 3.67−3.91 (4H), 3.38 (t, 1H, J = 5.4 Hz), 2.47−2.53 (t, 2H, J = 8.5 Hz), 1.40−1.74 (6H). The phosphonium salt was used for the next step without further purification.

Methyl 9-(2-Tetrahydropyranyloxy)nona-4(*Z*),7(*Z*)-dienoate (4). Method A: The Wittig reaction between phosphonium iodide **2** and aldehyde **3** was first performed following Corey's methodology.[23−26] To a solution of phosphonium iodide **2** (4.44 g, 7.95 mmol) in anhydrous THF (200 mL) at −78 °C was added *n*-butyllithium in hexane (4.72 mL of 1.6 M, 7.56 mmol, 0.95 equiv) dropwise. The resulting orange red solution was stirred at this temperature for another 2 h after which, HMPA (20 mL, 120 mmol, 15 equiv) was added dropwise. After an additional 30 min of stirring, methyl 4-oxobutanoate (**3**, 1.20 g, 10.4 mmol, 1.30 equiv) in THF (12 mL) was added over 45 min at −78 °C. The mixture was stirred for another 30 min and then was allowed to warm to 0 °C over 2 h. The reaction was quenched by addition of H_2O (20 mL), and solvent was removed by rotary evaporation. The residue was redissolved

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(41) Zhang, L.; Bi, Y.; Yu, F.; Menzia, G.; Cook, J. M. *Heterocycle* **1992**, *34*, 517−547.

in H_2O (50 mL) and then extracted with ether. The combined organic layers were washed with H_2O, 10% $Na_2S_2O_3$ solution, and brine and then dried. Solvent was removed by rotary evaporation under reduced pressure. The crude product was flash chromatographed on silica gel with 25% ethyl acetate in hexanes (R_f = 0.32) to deliver the desired product **4** (1.45 g, 68% based on phosphonium iodide). **Method B:** The Wittig reaction was also carried out with by another method.[22,27,28] To a solution of phosphonium iodide **2** (1.22 g, 2.87 mmol) in anhydrous THF (40 mL) at −78 °C was added lithium hexamethyldisilazide (2.58 mL of 1.0 M, 2.58 mmol, 0.90 equiv) dropwise. Then, after 2 h of stirring at this temperature, HMPA (6.5 mL) was added dropwise. After an additional 30 min of stirring at −78 °C, methyl 4-oxobutanoate (**3**, 433 mg, 3.73 mmol, 1.30 equiv) in THF (5 mL) was added into the red reaction mixture over 45 min at −78 °C. The mixture was then stirred for another 30 min at −78 °C and then allowed to warm to 0 °C over 2 h. The reaction was quenched by addition of saturated aqueous NH_4Cl (20 mL). The workup and purification were the same as for method A and gave **4** (423 mg, 55% yield based on phosphonium iodide): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.51−5.57 (2H, C7, C8−H), 5.35−5.38 (2H, C4, C5−H), 4.61 (t, 1H, C2′−H), 4.25 (dd, 1H, J = 6.0 Hz, J = 12.4 Hz, C9−Ha), 4.08 (dd, 1H, J = 6.0 Hz, J = 12.2 Hz, C9−Hb), 3.85 (1H, C6′-Ha), 3.65 (s, 3H, OCH_3), 3.49 (1H, C6′-Hb), 2.82−2.86 (dd, 2H, J = 5.8 Hz, J = 5.8 Hz, C6−H), 2.33−2.37 (4H, C2, C3−H), 1.48−1.83 (6H, C3′, C4′, C5′−H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 173.49, 131.36, 128.85, 128.20, 126.35, 97.87, 62.61, 62.22, 51.56, 33.95, 30.66, 25.88, 25.48, 22.77, 19.51; HRMS (20 eV) *m/z* calcd for $C_{15}H_{24}O_4$ (M^+) 268.1674, *m/z* found 268.1687.

Methyl 9-Hydroxynona-4(*Z*),7(*Z*)-dienoate (5). A solution of THP ether **4** (795 mg, 2.97 mmol) and PPTS[42] (74.8 mg, 0.297 mmol, 0.1 equiv) in methanol (24 mL) was stirred at 38 °C for 11 h (monitored by TLC for the disappearance of the THP ether). The solvent was removed by rotary evaporation under reduced pressure, and the residue was chromatographed on a silica gel column with 50% ethyl acetate in hexanes to afford the pure alcohol **5** (536 mg, 98% yield); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.64 (dt, 1H, J = 4.0 Hz, J = 8.4 Hz, C8−H), 5.37−5.47 (3H, C7, C4, C5−H), 4.23 (d, 2H, J = 6.6 Hz, C9−H), 3.68 (s, 3H, OCH_3), 2.84−2.87 (dd, 2H, J = 5.3 Hz, J = 5.6 Hz, C6−H), 2.36−2.40 (4H, C2, C3−H), 1.67 (s, 1H, −OH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 173.75, 130.42, 128.84 (2C), 128.26, 58.37, 51.67, 33.86, 25.78, 22.79; HRMS (20 eV) *m/z* calcd for $C_{10}H_{16}O_3$ (M^+) 184.1099, *m/z* found 268.1082.

Methyl 9-Bromonona-4(*Z*),7(*Z*)-dienoate (6). To a magnetically stirred solution of alcohol **5** (534 mg, 2.90 mmol) in anhydrous CH_2Cl_2 (60 mL) at 0 °C was added CBr_4 (1.16 g, 3.48 mmol, 1.2 equiv) and DIPHOS[43] (1.39 g, 3.48 mmol, 1.2 equiv) portionwise over 15 min. The reaction mixture was stirred at 0 °C for 1 h and then allowed to stir at room temperature for another 1.5 h (monitoring disappearance of alcohol by TLC). The solvent was removed by rotary evaporation under reduced pressure. The residue was dissolved in pentane. The suspension was filtered, washed with more pentane, and concentrated by rotary evaporation to give the crude product, which was purified by flash chromatography on a silica gel column with 5% ethyl acetate in hexanes to give the bromide **6** (688 mg, 96%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.73 (dt, 1H, J = 4.0 Hz, J = 8.6 Hz, C8−H), 5.55 (dt, 1H, J = 4.4 Hz, J = 8.6 Hz, C7−H), 5.38−5.42 (2H, C4, C5−H), 4.01 (d, 2H, J = 8.4 Hz, C9−H), 3.67 (s, 3H, OCH_3), 2.88−2.91 (dd, 2H, J = 5.2 Hz, J = 5.4 Hz, C6−H), 2.36−2.43 (4H, C2, C3−H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 173.46, 133.56, 128.95, 127.77, 125.67, 51.62, 33.89, 26.93, 25.27, 22.81; HRMS (20 eV) *m/z* calcd for $C_{10}H_{15}BrO_2$ (M^+) 246.0255, *m/z* found 246.0254.

Methyl 10-(Diethylphosphono)-11-oxododeca-4(*Z*),7-(*Z*)-dienoate (8). Alkyl phosphonate **8** was prepared by alkylation of diethyl phosphonoacetone (**7**).[20,21,29] To a magnetically stirred suspension of sodium hydride (138 mg, 95% purity, 5.44 mmol, 2.0 equiv) in anhydrous THF (14 mL) was added diethyl phosphonoacetone (**7**, 1.85 g, 9.52 mmol, 3.5 equiv) at 0 °C. After 1 h of stirring at 0 °C and 2 h at room temperature, methyl 9-bromonona-4(*Z*),7(*Z*)-dienoate (**6**, 673 mg, 2.72 mmol) in THF (1 mL) was added dropwise at 0 °C. The reaction mixture was warmed to room temperature and allowed to stir in the dark for 24 h. The solvent was removed by rotary evaporation, and water (5 mL) was added to the residue. The aqueous mixture was extracted with ethyl acetate. The combined ethyl acetate extracts were washed once with brine, dried, and concentrated under reduced pressure. The crude product was purified by chromatography on silica gel with 75% ethyl acetate in hexanes giving **8** (882 mg, 90% yield based on bromide **6**): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.43−5.35 (3H, C5, C7, C8−H), 5.26 (dt, 1H, J = 4.4 Hz, J = 8.6 Hz, C4−H), 4.08−4.19 (q, 4H, J = 8.3 Hz, OCH_2CH_3), 3.67 (s, 3H, OCH_3), 3.19 (ddd, 1H, J = 22.3 Hz, J = 9.8 Hz, J = 4.2 Hz, C10−H), 2.72−2.85 (3H, C6, C9−Ha), 2.50 (1H, C9−Hb), 2.29−2.38 (7H, C2, C3, C12−H), 1.28−1.36 (t, 6H, J = 7.0 Hz, OCH_2CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 203.09, 173.43, 130.38, 128.83, 128.32, 126.30 and 126.09 (split), 62.79 and 62.70 and 62.56 and 62.48 (split), 53.45 (d, J = 123.1 Hz) 51.49, 33.92, 31.46, 25.52, 24.36, 24.31, 22.75, 16.39 and 16.32; HRMS (20 eV) *m/z* calcd for $C_{17}H_{29}O_6P$ (M^+) 360.1701, *m/z* found 360.1702.

Methyl 10-Acetyl-12(*S*),13-isopropylidenedioxy-4(*Z*),7-(*Z*),10(*Z*/*E*)-tridecatrienoates (10E and 10Z). A magnetically stirred suspension of sodium hydride (49 mg, 95%, 1.93 mmol, 1.30 equiv) in anhydrous THF (2.5 mL) was cooled to −5 °C. The β-ketophosphonate **8** (534 mg, 1.48 mmol, 1.0 equiv) in anhydrous THF (2.5 mL) was added dropwise over 15 min. Stirring was continued at this temperature for 4 h. Then freshly prepared isopropylidene-D-glyceraldehyde (**9**, 244 mg, 1.78 mmol, 1.20 equiv, 95% purity) in anhydrous THF (1.5 mL) was added over 10 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 (5 mL) was added to the resulting brown oily, gummy residue. The aqueous mixture was extracted with diethyl ether. The combined organic extracts were washed with water, dried (anhydrous $MgSO_4$), and filtered. Solvent was removed under reduced pressure. The crude product was flash chromatographed on a silica gel column, eluting with 20% ethyl acetate in hexanes (v/v), furnishing a mixture of enones **10E** and **10Z** (R_f = 0.25, 324 mg, 65% based on β-ketophosphonate **8**). The **10Z** and **10E** isomers (1:8, respectively) were used without separation for the next reaction. Pure major isomer **10E** and minor isomer **10Z** were isolated by the HPLC using 20% ethyl acetate in hexane as eluant with a Whatman Partisil 10 column (4.6 mm ID × 25 cm) at a flow rate of 1.0 mL/min (retention time 18.6 min for **10Z**, 21.0 min for **10E**). **10Z** (minor isomer): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.78 (d, 1H, J = 8.3 Hz, C11−H), 5.53 (1H, C8−H), 5.32−5.44 (3H, C4, C5, C7−H), 4.90 (dd, 1H, J = 6.4, 6.8 Hz, C12−H), 4.30 (dd, 1H, J = 8.4, 6.8 Hz, C13−Ha), 3.67 (s, 3H, $-OCH_3$), 3.56 (dd, 1H, J = 8.4, 6.8 Hz, C13−Hb), 3.09 (d, 2H, J = 7.2 Hz, C9−H), 2.83 (2H, C6−H), 2.33−2.41 (4H, C2, C3−H), 2.26 (s, 3H, acetyl methyl), 1.44 (s, 3H, isopropylidene methyl), 1.36 (s, 3H, isopropylidene methyl); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 201.96 (acetyl carbonyl), 173.50 (C-1), 140.84 (C-10), 137.45 (C-11), 130.80 (C-8), 128.58 (C-7), 128.42 (C-5), 125.75 (C-4), 109.54 (acetonide carbon, $C(Me)_2$), 74.07, 69.65, 51.61, 33.92, 31.61, 29.26, 26.65, 25.65, 25.53, 22.85. **10E** (major isomer): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.54 (d, 1H, J = 8.2 Hz, C11−H), 5.33−5.43 (3H, C5, C7, C8−H), 5.16 (1H, C4−H), 4.93 (dd, 1H, J = 6.4, 6.2 Hz, C12−H), 4.19 (dd, 1H, J = 8.2, 6.2 Hz, C13−Ha), 3.68 (s, 3H, $-OCH_3$), 3.66 (dd, 1H, J = 8.2, 6.2 Hz, C13−Hb), 3.13 (dd, 1H, J = 6.2, 13.8 Hz, C9−Ha), 3.08 (dd, 1H, J = 6.2, 13.8 Hz, C9−Hb), 2.91 (2H, C6−H), 2.37−2.43 (4H, C2, C3−H), 2.35 (s, 3H, acetyl methyl), 1.48 (s, 3H, isopropylidene methyl), 1.42 (s, 3H, isopropylidene methyl); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 198.54 (acetyl carbonyl), 173.51 (C-1), 142.61 (C-10), 140.03 (C-11), 128.86 (C-7,

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C-8), 128.22 (C-5), 127.08 (C-4), 110.06 (acetonide carbon, $C(Me)_2$), 72.86, 69.00, 51.57, 33.98, 26.70, 25.80 (2C overlap), 25.70, 24.28, 22.84; HRMS (20 eV) *m/z* calcd for $C_{19}H_{28}O_5^+$ (M^+) 336.1937, *m/z* found 336.1940.

1,1-Diethoxyhex-3-yne (12). To a solution of 1,1-diethoxybut-3-yne (**11**, 2.79 g, 20 mmol) in anhydrous THF (30 mL) was added *n*-BuLi (13.3 mL of 1.8 M, 24 mmol, 1.2 equiv) in hexanes at −78 °C. The solution was kept at this temperature for 1 h and was then allowed to warm to −30 °C over 3 h. The solution was then cooled to −78 °C. Ethyl iodide (18.7 g, 9.6 mL, 120 mmol, 6.0 equiv) and HMPA (7.16 g, 7.0 mL, 40 mmol, 2.0 equiv) were added dropwise. A few milligrams of $CaCO_3$ were also added. The mixture was kept stirring at −78 °C for another 6 h. Then it was allowed to warm to room temperature and stirred at room temperature for 60 h. The reaction was quenched by adding water (10 mL). The resulting mixture was extracted with ether. The combined organic layer was washed extensively with water, 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 the desired 1,1-diethoxyhex-3-yne (**12**, bp 58−62 °C at 2.0 mmHg, 3.33 g, 98%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.59 (t, 1H, J = 7.8 Hz, C1−H), 3.62−3.70 (q, 2H, J = 7.0 Hz, $-OCH_2$a), 3.51−3.59 (q, 2H, J = 7.0 Hz, $-OCH_2$b), 2.45−2.49 (d, 2H, J = 7.8 Hz, C2−H), 2.13−2.17 (q, 2H, J = 7.5 Hz, C5−H), 1.20 (t, 6H, J = 7.0 Hz, $-OCH_2CH_3$), 1.10 (t, 3H, J = 7.5 Hz, C6−H); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 101.30, 83.29, 74.52, 61.77, 25.05, 15.47, 14.12, 12.48; HRMS (20 eV) *m/z* calcd for $C_{10}H_{16}O_3$ ($M^+ - OCH_2CH_3$) 125.0966, *m/z* found 125.0967.

1,1-Diethoxyhex-3-ene (13). To an ice-cooled flask containing Cp_2TiCl_2[44] (40 mg, 4.0 mg/mmol alkyne) was added dropwise i-BuMgBr (4.62 mL of 1.9 M, 8.778 mmol, 1.40 equiv) in dry ether. The reaction mixture was stirred at 0 °C for 45 min. Then 1,1-diethoxyhex-3-yne (**12**, 1.067 g, 6.27 mmol) was added dropwise. The cooling bath was removed, and stirring was continued for 48 h at room temperature. The reaction mixture was slowly poured into an ice-cooled aqueous solution of NH_4Cl with vigorous stirring. The organic layer was separated, and the aqueous layer was extracted with ether. The combined organic layer was washed with brine and dried, and solvent was removed under reduced pressure by rotary evaporation. The crude product was distilled under reduced pressure to furnish 1,1-diethoxyhex-3-ene (**13**, bp 40−43 °C at 1.0 mmHg, 884 mg, 82% based on alkyne): ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.45 (dt, 1H, J = 7.8, 8.2 Hz, C3−H), 5.33 (dt, J = 7.8, 7.8 Hz, C4−H), 4.52 (t, 1H, J = 7.8 Hz, C1−H), 3.57−3.67 (q, 2H, J = 7.0 Hz, $-OCH_2$), 3.43−3.53 (q, 2H, J = 7.0 Hz, $-OCH_2$), 2.32−2.37 (dd, 2H, J = 7.8, 7.8 Hz, C2−H), 2.13−2.17 (qt, 2H, J = 7.5, 7.8 Hz, C5−H), 1.73 (t, 6H, J = 7.0 Hz, $-OCH_2CH_3$), 0.94 (t, 3H, J = 7.5 Hz, C6−H); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 133.91, 123.16, 102.62, 61.13, 31.93, 20.73, 15.27, 14.12; δ HRMS (20 eV) *m/z* calcd for $C_{10}H_{16}O_3$ (M^+) 172.1463, *m/z* found 172.1445.

3-Hydroxy-5-octen-1-yne (16). A solution of 1,1-diethoxyhex-3-ene (**13**, 516 mg, 3.0 mmol) containing a few milligrams of 2,5-di-*tert*-butylhydroquinone (DBHQ) in acetic acid/water[45] (4/1, v/v, 15 mL) was stirred at room temperature for 1 h, monitoring the disappearance of acetal by TLC. The reaction mixture was cooled in ice bath and was neutralized by slowly adding solid $NaHCO_3$ with vigorous stirring. The neutralized aqueous layer was extracted with ether (3 × 25 mL) and dried over anhydrous K_2CO_3. The solvent was carefully removed under reduced pressure (> 80 mmHg) by rotary evaporation to provide the (*Z*)-3-hexeneal (**14**) free of isomerized enal or starting acetal **13**. Because of its instability, this aldehyde was used immediately and without purification for the next step. To an ice-cooled solution of ethynylmagnesium bromide (0.5 M, 8.28 mL, 1.38 equiv) in THF was added dropwise the above freshly made aldehyde **14** in THF (1.0 mL). The reaction mixture was allowed to warm to temperature and stirred overnight. Cold saturated aqueous NH_4Cl (10 mL) was added to quench the reaction. The organic layer was extracted with ether, and the combined organic layers were washed with brine, dried, and concentrated under reduced pressure by rotary evaporation. The crude product was purified by flash chromatography on silica gel with 20% ethyl acetate in hexanes (R_f = 0.20) as eluant to furnish 3-hydroxy-5-octen-1-yne (**16**, 246 mg, 66% based on the starting acetal **13**): ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.64 (dt, 1H, J = 7.8, 8.2 Hz, C5−H), 5.46 (dt, J = 8.2, 7.8 Hz, C6−H), 4.42 (t, 1H, J = 7.8 Hz, C3−H), 2.51 (dd, 2H, J = 7.8, 7.8 Hz, C4−H), 2.47 (s, 1H, C1−H), 2.05−2.15 (qt, 2H, J = 7.5, 7.8 Hz, C7−H), 0.99 (t, 3H, J = 7.5 Hz, C8−H); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 136.03, 122.49, 84.57, 72.98, 61.78, 35.71, 20.80, 14.21; HRMS (20 eV) *m/z* calcd for $C_{10}H_{16}O_3$ ($M^+ - H$) 123.0810, *m/z* found 123.0812.

3-[(*tert*-Butyldimethylsilyl)oxy]-5(*Z*)-octen-1-yne (17). Alcohol **16** was converted to the corresponding TBDMS ether using Corey's methodology.[46] A solution of 3-hydroxy-5-octen-1-yne (**16**, 125 mg, 1.0 mmol), *tert*-butyldimethylsilyl chloride (226 mg, 1.50 mmol, 1.5 equiv), and imidazole (204 mg, 3.0 mmol, 3.0 equiv) in dry DMF (6 mL) was stirred at room temperature, monitoring the disappearance of alcohol **16** by TLC. After 20 h the mixture was poured into a separatory funnel containing hexanes and saturated aqueous $NaHCO_3$. The aqueous layer was extracted with hexanes. The combined organic layers were dried (anhydrous $MgSO_4$) and concentrated by rotary evaporation under reduced pressure to afford an oily residue that was purified by flash chromatography on silica gel with hexanes (R_f = 0.40) to afford the desired silyl ether, 3-[(*tert*-butyldimethylsilyl)oxy]-5(*Z*)-octen-1-yne (**17**, 234 mg, 98%) as a clear colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.64 (dt, 1H, J = 7.8, 8.2 Hz, C5−H), 5.42 (dt, J = 8.2, 7.8 Hz, C6−H), 4.34 (t, 1H, J = 6.8 Hz, C3−H), 2.43 (dd, 2H, J = 6.8, 7.8 Hz, C4−H), 2.39 (s, 1H, C1−H), 2.05−2.10 (qt, 2H, J = 7.5, 7.8 Hz, C7−H), 0.99 (t, 3H, J = 7.5 Hz, C8−H)0.0.90−0.95 (9H), 0.12−0.14 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 134.49, 123.63, 85.40, 72.09, 62.83, 36.51, 25.79, 20.81, 18.28, 14.26, −4.62, −5.02 (split); HRMS (20 eV) *m/z* calcd for $C_{14}H_{26}OSi$ (M^+) 238.1753, *m/z* found 238.1742.

3-[(*tert*-Butyldimethylsilyl)oxy]-1-(tri-*n*-butylstannyl)-1(*E*),5(*Z*)-octadiene (18E,Z) and the *E*,*E* isomer (18E,E). To a stirred mixture of 3-[(tertbutyldimethyl-silyl)oxy]-1-octyne (**17**, 1.38 g, 5.80 mmol) and azobis(isobutyonitrile) (AIBN, 25 mg) was added tri-*n*-butylstannane (1.69 g, 1.56 mL, 5.80 mmol, 1.0 equiv) with an airtight syringe under an argon atmosphere. The mixture was heated at 80 °C, stirred for 2 h, and then cooled to room temperature. The product mixture was rapidly flash chromatographed on a silica gel column with hexanes to produce an inseparable mixture of **Z**,**E** and **E**,**E** isomers (1:3 ratio according to ^{1}H NMR analysis, 2.33 g, 76%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.04 (d, 1H, J = 19.2 Hz, C1−H), 5.92 (dd, H, J = 19.2, 5.6 Hz, C2−H), 5.40−5.46 (1H, C4−H), 5.33−5.39 (1H, C5−H), 4.03−4.09 (1H, C3−H), 2.10−2.19 (dd, J = 5.6, 6.6 Hz, 2H, C4−H), 1.99−2.07 (qt, J = 6.6, 6.8 Hz, 2H, C7−H), 1.20−1.60 (18H, $-CH_2CH_2CH_2$-), 0.66−1.10 (21H, n-Bu methyls, t-Bu methyls, and C_2H_5 methyl), 0.04−0.05 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 151.39*, 151.22, 134.30*, 133.14, 126.60, 126.36*, 125.55*, 125.10, 76.81*, 76.61, 41.65*, 36.12, 29.15, 29.01*, 27.65*, 27.29, 25.95, 25.70*, 20.77, 20.77*, 18.40, 18.40*, 14.31, 14.31*, 13.74, 13.74*, 10.00*, 9.48, −4.39, −4.37*, −4.72, −4.73* (unmarked is **E**,**Z** isomer, asterisked is **E**,**E** isomer).

1-(Tri-*n*-butylstannyl)-1(E),5(*Z*)-octadien-3-ol (19). The required aldehyde, 3(*Z*)-hexenal (**14**), was freshly prepared from 1,1-diethoxy-3(*Z*)-hexene (1.032 g, 6.00 mmol, 1.0 equiv) as described above for the synthesis of 3-hydroxy-5-octen-1-yne (**16**). To a solution of *trans*-bis(tri-*n*-butylstannyl)ethylene (**19**, 7.27 g, 12.0 mmol, 2.0 equiv) in THF (20 mL) at −78 °C was slowly added *n*-BuLi (9.05 mL, 1.23 M in hexane, 11.1 mmol, 1.85 equiv). After 2 h of stirring at −60 °C, 3(*Z*)-hexenal (**14**, 610 mg) was added. The solution was stirred for 2 h at 0 °C. The reaction was then quenched with saturated aqueous

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NH_4Cl solution (5 mL). The mixture was extracted with hexanes. The combined extracts were dried (anhydrous $MgSO_4$) and concentrated under reduced pressure to afford a colorless oily residue (2.5 g). This oil was purified by flash chromatography on silica gel first with hexanes (2 column volumes), which removes the nonpolar side products Bu_4Sn and $Bu_3Sn-CH=CH_2$, and then with 2.5% ethyl acetate in hexanes ($R_f = 0.33$) to provide vinylstannane alcohol **19** (1.70 g). The alcohol **19** was further purified by preparative HPLC with 2.0% ethyl acetate/hexane using a Whatman Partisil 10, M9 column (9.4 mm ID × 50 cm) at a flow rate of 2 mL/min (retention time = 22 min) to provide pure 1-(tri-*n*-butylstannyl)-1(*E*),5(*Z*)-octadien-3-ol (**19**, 1.60 g, 64% based on acetal precursor **13** of aldehyde **14**): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.16 (d, H, $J = 19.2$ Hz, C1−H), 6.02 (dd, H, $J = 19.2$, 6.0 Hz, C2−H), 5.54−5.60 (m, 1H, C5−H), 5.32−5.52 (m, 1H, C6−H), 4.08−4.13 (m, 1H, C3−H), 2.23−2.34 (dd, $J = 6.0$, 6.6 Hz, 2H, C4−H), 2.03−2.14 (q, $J = 6.8$ Hz, 2H, C7−H), 1.66−1.72 (broad s, 1H, −OH), 1.20−1.60 (18H, $-CH_2CH_2CH_2-$), 0.85−1.11 (t, $J = 7.2$ Hz, 9H, *n*-Bu methyls and 3H, C_2H_5 methyl); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 150.19, 134.97, 127.84, 123.99, 74.69, 35.03, 29.10, 27.29, 20.77, 14.28, 13.72, 9.49; HRMS (20 eV) *m*/*z* calcd for $C_{19}H_{37}O^{120}Sn$ ($M^+ - C_4H_9$) 357.1393, *m*/*z* found 357.1363.

3-[(*tert*-Butyldimethylsilyl)oxy]-1-(tri-*n*-butylstannyl)-1(*E*),5(*Z*)-octadiene (18E,Z). Alcohol **20** was converted to the corresponding TBDMS ether using Corey's methodology.[46] A solution of 1-(tri-*n*-butylstannyl)-1(*E*),5(*Z*)-octadien-3-ol (**20**, 936 mg, 2.18 mmol, 1.0 equiv), *tert*-butyldimethylsilyl chloride (493 mg, 3.27 mmol, 1.5 equiv) and imidazole (446 mg, 6.55 mmol, 3.0 equiv) in dry DMF (13.0 mL) was stirred at room temperature for 15 h, monitoring the disappearance of alcohol by TLC, and then poured into a mixture of hexanes and saturated aqueous $NaHCO_3$. The aqueous layer was extracted with hexanes. The combined organic layers were dried (anhydrous $MgSO_4$) and concentrated under reduced pressure by rotary evaporation to afford an oily residue that was purified by flash chromatography on silica gel with hexanes ($R_f = 0.40$) to deliver the desired silyl ether, 3-[(*tert*-butyldimethylsilyl)-oxy]-1-(tri-*n*-butylstannyl)-1(*E*),5(*Z*)-octadiene (**18**, 1.108 g, 96%), as a clear colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.04 (d, 1H, $J = 19.2$ Hz, C1−H), 5.92 (dd, H, $J = 19.2$, 5.6 Hz, C2−H), 5.40−5.46 (m, H, C4−H), 5.33−5.39 (m, H, C5−H), 4.03−4.09 (1H, C3−H), 2.10−2.19 (dd, $J = 5.6$, 6.6 Hz, 2H, C4−H), 1.99−2.07 (qt, $J = 6.6$, 6.8 Hz, 2H, C7−H), 1.20−1.60 (18H, $-CH_2CH_2CH_2-$), 0.66−1.10 (21H, *n*-Bu methyls ($J = 7.2$ Hz), *t*-Bu methyls, and CH_2CH_3 methyl), 0.04−0.05 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 151.22, 133.15, 126.61, 125.11, 76.56, 36.12, 29.15, 27.30, 25.96, 20.77, 18.37, 14.31, 13.75, 9.48, −4.39, −4.72; HRMS (20 eV) *m*/*z* calcd for $C_{25}H_{51}OSi^{120}Sn$ ($M^+ - C_4H_9$) 471.2258, *m*/*z* found 471.2283.

Conjugate Addition of a Higher Order Vinyl Cyanocuprate (21) to a γ-Alkoxy Enone (18). Construction of the 17-isoLGE$_4$ (24) Skeleton. The vinylcuprate reagent **21** was generated in situ by transmetalation of vinylstannane **18** with dimethyl cyanocuprate as described previously.[33] Copper cyanide (14 mg, 0.15 mmol, 1.07 equiv, flame dried under argon) in THF (1.0 mL) was treated with methyllithium (0.33 mmol, 235 μL of 1.40 M, 2.36 equiv) at 0 °C. The cooling bath was removed, and vinylstannane **18** (80 mg, 0.15 mmol, 1.07 equiv) in THF (800 μL) was added. After 2 h at room temperature, the reaction flask was cooled to −78 °C with a dry ice−acetone bath. Enone **10** (*Z*/*E* = 1:8, 46 mg, 0.14 mmol, 1.0 equiv) in THF (1.0 mL) was added rapidly with a syringe. After 30 min of stirring at −78 °C, the reaction was allowed to warm to −35 °C over another 30 min and then quenched by addition of saturated aqueous ammonium chloride and ammonium hydroxide (9:1, v/v, 1 mL). The resulting mixture was extracted with diethyl ether. The combined organic extracts were 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*-butylstannylmethane ($R_f = 0.82$), a diastereomeric mixture of conjugate addition products **22** ($R_f = 0.18$ and 0.13) and unreacted enone **10** ($R_f = 0.06$). The unreacted enone **10** that was recovered was almost exclusively the *E* isomer. The crude product (856 mg) was purified by flash chromatography on silica gel with 8% ethyl acetate in hexanes to afford the erythro isomers **22SS** and **22SR** (2.5 mg), mixtures of erythro and threo isomers (13.0 mg), and threo isomers **22RS** and **22RR** (13.9 mg). Further elution of the column with 15% ethyl acetate in hexanes delivered unreacted enones (14.2 mg). The overall yield (53%) of conjugate addition products was calculated based on enone reacted (32 mg). The mixture of erythro and threo isomers was further purified by HPLC on a Whatman Partisil 10 column (4.6 mm ID × 25 cm) at a flow rate of 1.0 mL/min (retention times, 18.5 and 21.8 min) with 8% ethyl acetate in hexanes as eluant. The ratio of threo to erythro isomers was finally determined as 68:32. Threo isomers (**22SR and 22SS**): ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.20−5.62 (8H, −CH=CH−), 4.09 (1H, C-14H), 3.98−3.90 (2H, C20−H, C21−Ha), 3.68 (s, 3H, $-OCH_3$), 3.55−3.64 (1H, C21−Hb), 2.70−2.77 (3H, C10−H, C6−H), 2.54−2.57 (1H, C11−H), 2.33−2.37 (4H, C2−H, C3−H), 2.17−2.31 (3H, C9−Ha, C15−H), 2.13 (s, 3H, CH_3CO-), 1.97−2.07 (3H, C9−Hb, C18−H), 1.31−1.38 (6H, isopropylidene methyls), 0.95 (t, 3H, $J = 7.5$ Hz, terminal CH_3), 0.89 (s, 9H, t-Bu), 0.02−0.05 (6H, $Si(CH_3)_2$). Erythro isomers (**22RR and 22RS**): ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.28−5.52 (8H, −CH=CH-), 4.10 (1H, C-14H), 3.88−3.96 (2H, C20−H, C21−Ha), 3.68 (s, 3H, $-OCH_3$), 3.51−3.58 (1H, C21−Hb), 3.03−3.10 (1H, C10−H), 2.76−2.79 (2H, C6−H), 2.35−2.43 (4H, C2−H, C3−H), 2.22−2.33 (4H, C9−Ha, C11−H, C15−H), 2.20 (s, 3H, CH_3CO-), 1.98−2.15 (3H, C9−Hb, C18−H), 1.42 (s, 3H, isopropylidene methyl), 1.34 (s, 3H, isopropylidene methyl), 0.95 (t, 3H, $J = 7.6$ Hz, terminal CH_3), 0.90 (s, 9H, *t*-Bu), 0.04−0.05 (6H, $Si(CH_3)_2$); HRMS (20 eV) *m*/*z* calcd for $C_{31}H_{55}O_6Si$ ($M^+ - CH_3$) 561.3611, *m*/*z* found 562.3598.

Hydrolysis of Methyl Esters 22. Hydrolysis of methyl esters **22** under mild conditions was carried out by a modified literature method.[27] A aqueous solution of lithium hydroxide (1.0 M, 85 μL, 5.0 equiv) and THF (425 μL) with a few milligrams of butylated hydroxytoluene (BHT) was added to a 68:32 mixture of threo methyl esters (**22SS**, **22SR**) and erythro methyl esters (**22RS**, **22RR**) (10 mg, 0.017 mmol). The mixture was stirred at room temperature for 13 h whereupon TLC analysis with 20% ethyl acetate in hexanes showed the complete disappearance of starting esters ($R_f = 0.42$) and a new, more polar spot ($R_f = 0.13$). The reaction mixture was carefully acidified by adding saturated aqueous NH_4Cl/water (1/1, v/v, 1.0 mL) and extracted with ethyl acetate. The combined organic extracts were dried (anhydrous $MgSO_4$), filtered, and concentrated by rotary evaporation. The crude product was purified on a silica gel column using 10% ethyl acetate in hexanes to remove the nonpolar byproducts and then with 35% ethyl acetate ($R_f = 0.25$) as eluant to afford the desired acid **23** (7.8 mg, 82%). The same mixture of isomers was obtained using either pure threo methyl esters (**22SS**, **22SR**) or erythro methyl esters (**22RS**, **22RR**). Thus, owing to epimerization at C-10, the same 30:70 ratio was seen for the integral area for the C-10 hydrogen resonance for erythro (δ 3.06) to threo (δ 2.75) diastereomers in the hydrolysis product regardless of the stereochemical composition of the starting ester. Acid **23**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.21−5.64 (8H, −CH=CH-), 4.01−4.14 (1H, C14−H), 3.88−3.97 (2H, C20−H, C21−Ha), 3.53−3.65 (1H, C-21Hb), 3.03−3.05 (0.3H, C10−H erythro), 2.67−2.78 (2.7H, C10−H threo, C6−H) 2.22−2.65 (8H, C2−H, C3−H, C9−Ha, C11−H, C15−H), 2.16 (s, 3H, CH_3CO-), 1.89−2.17 (3H, C9−Hb, C18−H), 1.26−1.41 (6H, isopropylidene methyls), 0.93−0.98 (apparent t, 3H, terminal CH_3), 0.89 (s, 9H, *t*-Bu), 0.03−0.05 (6H, $Si(CH_3)_2$); HRMS (20 eV) *m*/*z* calcd for $C_{31}H_{55}O_6Si$ ($M^+ - CH_3$) 562.3689, *m*/*z* found 562.3662.

17-isoLGE$_4$ (10-Acetyl-11-formyl-14-hydroxynonadeca-4(*Z*),7(*Z*),12(*E*),16(*Z*)-tetraenoic Acid). Simultaneous deprotection of the acetonide and TBDMS ether and subsequent oxidative cleavage of the vicinal diol was utilized for the synthesis of 17-isoLGE$_4$. Thus, a magnetically stirred solution of acid **23** (5.8 mg, 0.010 mmol) in acetic acid−water (268 μL, 2/1, v/v) containing BHT (1 mg) under argon was warmed to 40 °C. After 4 h of stirring, TLC analysis showed that the

resulting solution contained completely deprotected triol acid, $R_f = 0.15$ (20% 2-propanol/1.5% acetic acid in hexanes, v/v/v). This solution was added to sodium metaperiodate (2.8 mg, 0.013 mmol, 1.3 equiv) in acetone−water (1.32 mL, 3/7, v/v). After 1.8 h of stirring at room temperature, the reaction was quenched by addition of ethylene glycol (3.7 mg, 0.060 mmol, 6 equiv). After an additional 15 min at room temperature, the reaction mixture was transferred to a separatory funnel containing *freshly distilled* diethyl ether. The aqueous layer was removed and reextracted with *freshly distilled* diethyl ether. The combined organic extracts were washed extensively with water to remove oxidation byproducts. The diethyl ether extract was dried (anhydrous $MgSO_4$) and filtered, and *n*-heptane (20 mL) was added to the filtrate. The solvent volume was reduced to about 10 mL by rotary evaporation at 20 °C, and additional portions of *n*-heptane (20 mL) and diethyl ether (10 mL) were 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 3.8 mg (98% material balance) of a clear oil which was free of volatile byproducts and acetic acid. The purity of 17-isoLGE$_4$ was determined by the ratio of the integrated area of the aldehydic proton resonance (δ 9.48, d, $J = 6.0$ Hz, 1H) to the proton resonance at C14 (δ 4.10−4.30, 1H). To ensure 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 the Varian Gemini T_1 software program. For 17-isoLGE$_4$ in $CDCl_3$ at 20 °C the T_1 relaxation of aldehyde signal (δ 9.50, 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. The integrated aldehydic resonance was 45% of the expected area relative to that proton. Characteristic resonances of the major isomers and their assignments: δ 9.48 (d, $J = 6.0$ Hz, 1H, CHO), 5.22−5.88 (8H, olefinic protons), 4.10−4.30 (1H, C14−H), 3.58 (1H, C11−H), 3.02 (1H, C10−H), 2.64−2.94 (m, 2H of C6−H), 2.26 (s, 3H, $COCH_3$). This compound was further characterized by conversion to a bismethoxime derivative.

17-isoLGM$_4$ (10-(2-Aza-2-methoxy-1-methylvinyl)-11-(2-aza-2-methoxyvinyl)-14-hydroxynonadeca-4(*Z*),7(*Z*),12(*E*),16(*Z*)-tetraenoic Acid). A solution of freshly made 17-isoLGE$_4$ (11.0 mg, 45% purity) and methoxylamine hydrochloride (20 mg, 0.24 mmol) in *anhydrous* pyridine (250 μL) was stirred at room temperature for 15 h. Pyridine was evaporated under a stream of argon, and then water (1.0 mL) was added to the solid residue. The aqueous layer was extracted with ethyl acetate, and the combined organic extracts were dried with anhydrous $MgSO_4$ and concentrated by rotary evaporation. The crude product was chromatographed on silica gel with 75% ethyl acetate in hexanes ($R_f = 0.18$) to afford the desired methoxime derivative 17-isoLGM$_4$ (6.2 mg). The compound was purified further by HPLC using a Whatman Partisil column with 50% ethyl acetate in hexanes as eluant at a flow rate of 1.0 mL/min (retention time 7.6 min) to give the pure bismethoxime (4.60 mg, 81% yield based on pure 17-isoLGE$_4$): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.22 (dd, 1H, $J = 6.8$ Hz, C20−H), 5.21−5.72 (8H, −CH=CH−), 4.16−4.22 (1H, C14−H), 3.83 (s, 3H, $-OCH_3$), 3.79 (s, 3H, $-OCH_3$), 3.04−3.12 (apparent q, $J = 7.8$ Hz, C11−H), 2.82−2.96 (1H, C6−H), 2.66−2.77 (1H, C6−H), 2.00−2.52 (11H, C2−H, C3−H, C9−H, C10−H, C15−H, C18−H), 1.70−1.84 (s, 3H, $-CNCH_3$), 0.95−1.00 (t, 3H, $J = 7.4$ Hz, terminal methyl); HRMS (20 eV) *m*/*z* calcd for $C_{24}H_{38}N_2O_5$ (M^+) 434.2780, *m*/*z* found: 434.2778.

17-isoLGM$_4$-PFB ((2,3,4,5,6-Pentafluorophenyl)methyl 10-(2-Aza-2-methoxy-1-methylvinyl)-11-(2-aza-2-methoxyvinyl)-14-hydroxynonadeca-4(*Z*),7(*Z*), 12(*E*),16(*Z*)-tetraenoate). To a solution of 17-isoLGM$_4$ (3.5 mg, 0.008 mmol) and diisopropylamine (10 mg, 0.09 mmol) in anhydrous acetonitrile (350 μL) was added a solution of pentafluorobenzyl bromide (30 mg, 0.14 mmol) in acetonitrile (100 μL). The reaction was stirred at room temperature for 8 h, and then solvent was removed under reduced pressure by rotary evaporation. The crude product was purified by flash chromatography on silica gel with 30% ethyl acetate in hexanes ($R_f = 0.32$). The residue was further purified by HPLC using 30% ethyl acetate in hexanes as eluant on a Whatman Partisil column (1.0 mL/min; retention time, 11.4 min) to afford the desired ester 17-isoLGM$_4$-PFB (4.1 mg, 85%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.22 (dd, 1H, $J = 6.8$ Hz, C20−H), 5.23−5.72 (8H, −CH=CH−), 5.20 (s, 2H, $-OCH_2C_6F_5$), 4.14−4.18 (1H, C14−H), 3.82 (s, 3H, $-OCH_3$), 3.79 (s, 3H, $-OCH_3$), 3.04−3.12 (apparent q, $J = 7.8$ Hz, C11−H), 2.71−2.80 (2H, C6−H), 2.01−2.50 (11H, C2−H, C3−H, C9−H, C10−H, C15−H, C18−H), 1.73 (s, 3H, $-CNCH_3$), 0.94−0.98 (t, 3H, $J = 7.5$ Hz, terminal methyl); HRMS (20 eV) *m*/*z* calcd for $C_{31}H_{38}F_5N_2O_5$ (M^+) 614.2779, *m*/*z* found 614.2808.

17-isoLGM$_4$-TMS-PFB ((2,3,4,5,6-Pentafluorophenyl)methyl 10-(2-Aza-2-methoxy-1-methylvinyl)-11-(2-aza-2-methoxyvinyl)-14-hydroxynonadeca-4(*Z*),7(*Z*),12(*E*),16(*Z*)-tetraenoate). To a solution of 17-isoLGM$_4$-PFB (300 μg) in anhydrous DMF (25 μL) was added bis(trimethylsilyl)trifluoroacetamide (BTMSTFA, 75 μL), and the mixture was stirred by 37 °C for 25 min. The volatiles were evaporated by a stream of argon. The product was directly analyzed by HRMS to determine the characteristic ions (see Table 3) and confirm the structure.

Δ^{11}-17-isoLGM$_4$ (10-(2-Aza-2-methoxy-1-methylvinyl)-11-(2-aza-2-methoxyvinyl)-14-hydroxynonadeca-4(*Z*),7(*Z*),11(*E*),16(*Z*)-tetraenoic Acid). A solution of freshly made 17-isoLGE$_4$ (5.5 mg, 35% purity) and methoxylamine hydrochloride (10 mg, 0.12 mmol) in *wet* pyridine (150 μL) was stirred at room temperature for 24 h. Pyridine was evaporated under a stream of argon, and then water (1.0 mL) was added to the solid residue. The aqueous layer was extracted with ethyl acetate, the combined organic extracts were dried ($MgSO_4$), and solvents were removed by rotary evaporation. The crude product was chromatographed on silica gel with 75% ethyl acetate in hexanes ($R_f = 0.22$) to afford the isomerized bismethoxime Δ^{11}-17-isoLGM$_4$ (1.68 mg, 75% yield based on pure 17-isoLGE$_4$): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.62 (d, 1H, $J = 8.8$ Hz, C20−H), 5.92 (apparent q, 1H, $J = 12.8$ Hz, C12−H), 5.31−5.62 (6H, −CH=CH−), 3.83 (s, 3H, $-OCH_3$), 3.79 (s, 3H, $-OCH_3$), 3.62−3.78 (2H, C10, C14−H), 2.85−2.98 (1H, C6−H), 2.69−2.78 (1H, C6), 2.60−2.68 (1H, C9−H), 2.30−2.520 (7H, C2−H, C3−H, C9−H, C13−H), 2.18−2.20 (2H, C15−H), 2.01−2.12 (2H, C18−H), 1.81 (s, 3H, $-CNCH_3$), 0.95−1.00 (t, 3H, $J = 7.8$ Hz, terminal methyl); HRMS (20 eV) *m*/*z* calcd for $C_{24}H_{38}N_2O_5$ (M^+) 434.2780, *m*/*z* found 434.2776.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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