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An Epoxyisoprostane Is a Major Regulator of Endothelial Cell Function

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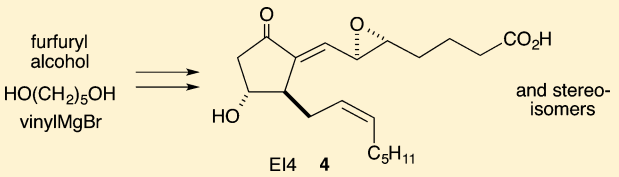
Supporting Information

ABSTRACT: The goal of these studies was to determine the effect of 5,6-epoxyisoprostane, EI, on human aortic endothelial cells (HAEC). EI can form as a phospholipase product of 1-palmitoyl-2-(5,6-epoxyisoprostane E2)-*sn*-glycero-3-phosphocholine, PEIPC, a proinflammatory molecule that accumulates in sites of inflammation where phospholipases are also increased. To determine the effect of EI on HAEC, we synthesized several stereoisomers of EI using a convergent approach from the individual optically pure building blocks, the epoxyaldehydes **5** and **6** and the bromoenones **14** and **16**. The desired stereoisomer of EI can be prepared from these materials in only six operations, and thus, large amounts of the product can be obtained. The trans/trans isomers had the most potent activity, suggesting specificity in the interaction of EI with the cell surface. EI has potent anti-inflammatory effects in HAEC. EI strongly inhibits the production of MCP-1, a major monocyte chemotactic factor, and either decreases or minimally increases the levels of 10 proinflammatory molecules increased by PEIPC. EI also strongly down-regulates the inflammatory effects of IL-1β, a major inflammatory cytokine. Thus EI, a hydrolytic product of PEIPC, has potent anti-inflammatory function.



INTRODUCTION

In vitro lipid oxidation products of 1-palmitoyl-2-arachidonoyl-*sn*-phosphatidylcholine (PAPC) have been shown to accumulate in oxidized lipoproteins, the membranes of cells exposed to oxidative stress, and in apoptotic and necrotic cells.[1−3] In vivo they have been shown to accumulate in atherosclerotic lesions and other sites of chronic inflammation.[2,4−6] One of the oxidation products that accumulates is 1-palmitoyl-2-(5,6-epoxyisoprostane E2)-*sn*-glycero-3-phosphocholine, PEIPC.[4] Using microarray analysis of human aortic endothelial cells (HAEC) from 147 donors, our group has shown that a 4 h treatment with oxidized 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine (Ox-PAPC) regulates more than 1000 genes. PEIPC regulates 80% of these genes. Some of the pathways activated are protective against cell death including increases in unfolded protein response (UPR), inhibition of DNA synthesis, and an increase in antioxidant genes.[7] PEIPC and Ox-PAPC also have proinflammatory effects, increasing the binding of monocytes to HAEC and increasing the production of chemotactic factors that cause monocytes to enter the vessel wall.[8−10] PEIPC is the most active oxidation product of Ox-PAPC with regard to effects on HAEC inflammatory molecules, being active in the low micromolar range.[7] Previous studies have demonstrated that phospholipases (PLA2) that can hydrolyze PEIPC[11] increase in atherosclerotic lesions and can release EI. In order to facilitate studies of the action of 5,6-epoxyisoprostane, EI, on HAEC and to facilitate the synthesis of PEIPC, we developed an efficient strategy for the synthesis of a series of epoxyisoprostane (EI) analogues. We previously demonstrated that four regioisomers of PEIPC can be formed during oxidation.[9] However, the isomer where the epoxide group was located in the 5,6-position was the most active and most readily formed during PAPC oxidation.[9] Therefore, we synthesized four optically pure stereoisomers of this EI for testing in our studies. Their ability to regulate HAEC function was then examined. We present evidence that EI has many functions in common with PEIPC and may be a useful analogue to identify pathways regulated by both agents. However, EI treatment of HAEC results in lower production of inflammatory cytokines than PEIPC, actually decreases constitutive levels of MCP-1, and inhibits the action of IL-1β, a cytokine highly increased in atherosclerotic lesions.

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RESULTS

Synthesis of EI Analogues. On the basis of our previous work on the synthesis of PEIPC,[12,13] we designed a more efficient synthetic route to produce EI4 **4** and its analogues EI1−EI3 (**1**−**3**, Figure 1). Since the *p*-methoxybenzyl (PMB)

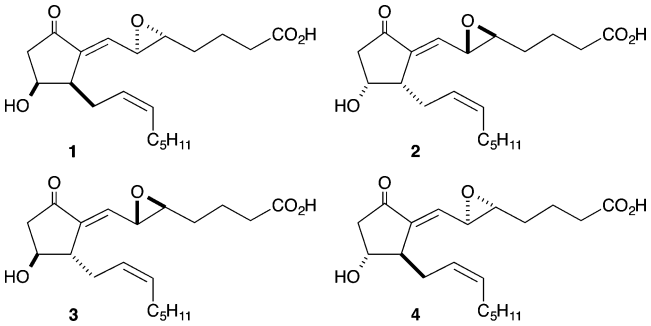
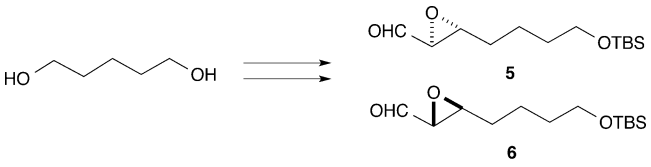


Figure 1. Structures of EI analogues.

ether was the key protecting group, because it was removable under nonbasic conditions, we decided to install it at an earlier stage than previously described. Then our previous developed triply convergent coupling strategy could be followed to achieve the synthesis of the EI analogues.

The components for the key coupling were prepared as follows. The optically pure epoxyaldehydes **5** and **6** were prepared by our previous synthetic route via initial selective protection of pentane-1,5-diol as the monosilyl ether (Scheme 1), followed by Swern oxidation to the aldehyde, Horner−

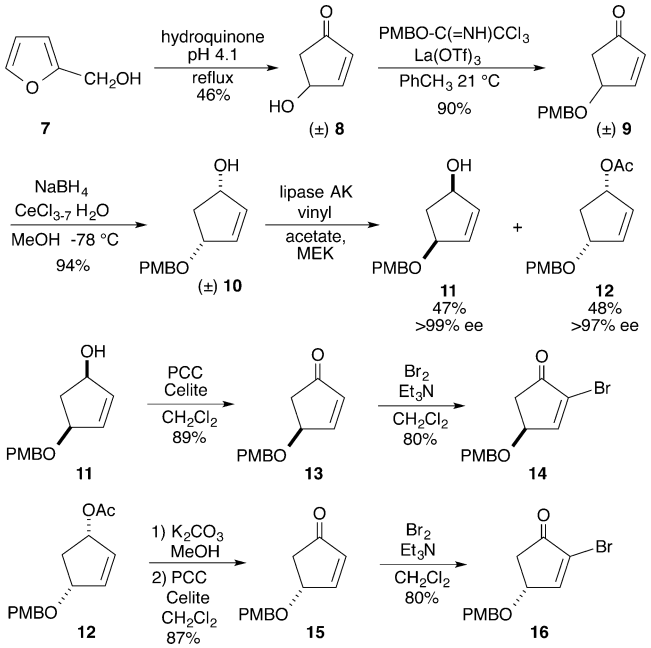
Scheme 1. Synthesis of Epoxyaldehydes 5 and 6



Wadsworth−Emmons reaction to give the *E*-enoate, and reduction to give the *E*-allylic alcohol. Subjection of this alcohol to Sharpless asymmetric epoxidation, with either L- or D-dialkyl tartrate, and final oxidation with Dess−Martin periodinane gave the optically pure epoxides **5** and **6**.[13]

The second key components were the desired optically pure intermediates **14** and **16**, which were prepared in an efficient, high-yielding six- or seven-step sequence (Scheme 2). The route featured an enzymatic resolution of the mono-PMB-protected 1,4-cyclopentenediol and La(OTf)$_3$-promoted PMB ether formation. The commercially available, inexpensive furfuryl alcohol **7** was converted into 4-hydroxycyclopentenone **8** in 46% yield by refluxing under mildly acidic conditions (pH 4.1).[14] Even though the yield was somewhat low, the ready availability of the starting materials and ease of synthesis made this the preferable method for preparing **8**. Our initial attempts to install the PMB ether protecting group using traditional methods of base and PMB chloride or bromide resulted in low yields (<40%). However, treatment of 4-hydroxycyclopentenone **8** with freshly prepared *p*-methoxybenzyl trichloroacetimidate in the presence of a catalytic amount of lanthanum triflate[15] in toluene at 21 °C gave the desired product **9** in 90% yield. Luche reduction[16] of the *α*,*β*-unsaturated ketone of **9** using sodium borohydride and cerium(III) chloride proceeded

Scheme 2. Synthesis of Pure Bromoenones 14 and 16



very selectively and gave, after flash column chromatography, only the cis-isomer **10** in 94% yield as a mixture of enantiomers. Enzymatic kinetic resolution of the racemic mono-PMB ether of cyclopent-1-ene-3,5-diol **10** was achieved using lipase AK with vinyl acetate in methyl ethyl ketone (MEK) as solvent. This enzyme-catalyzed transesterification produced both enantiomeric products in excellent yield and optical purity, namely, the 3*R*,5*S*-5-*p*-methoxybenzyloxycyclopent-1-en-3-ol **11** (47% yield, >99% ee) and the 3*S*,5*R*-5-*p*-methoxybenzyloxycyclopent-1-en-3-ol acetate **12** (48% yield, >97% ee). Thus, this is an improvement of our previous route[13] to produce such important, optically active oxygenated cyclopentene systems. The enzyme can be reused several times without loss of efficiency or selectivity. As far as we can tell, this is the first time an enzymatic kinetic resolution using lipase AK has been employed to separate a racemic mixture of the mono-PMB ether of cyclopent-1-ene-3,5-diol derivatives, although several other ethers have been used.[17] The conversion of the enantiomerically enriched cyclopentenol **11** to the desired *S*-2-bromo-4-arylmethoxycyclopentenone **14** was achieved in two steps, via oxidation to the enone **13** with pyridinium chlorochromate (PCC, 89%) followed by bromination in the presence of triethylamine to give **14** in 80% yield. The enantiomeric *R*-2-bromo-4-arylmethoxycyclopentenone **16** was prepared in three steps from **12** via deprotection of the acetate and PCC oxidation to give the enone **15** in 87% yield followed by bromination in the presence of base to give **16** in 80% yield.

With these two building blocks **14** and **16** in hand, we examined various processes, e.g., a silyl group transfer reaction, to introduce substituents on the enone. The silyl group transfer of the enone **14** with the silyl ketene acetal **17** produced an inseparable 1:1 mixture of the two silyl enol ether esters **18** (Scheme 3). The analogous reaction catalyzed by either HgI_2 and SmI_2 gave lower yields (<20%) than the reaction using $TiCl_4$, which gave the highest yield (77%).[18] Selective reduction of the ester of **18** to the aldehyde with diisobutylaluminum hydride (DIBAL) at −78 °C allowed for the easy chromatographic separation of the trans and cis disubstituted bromoenol

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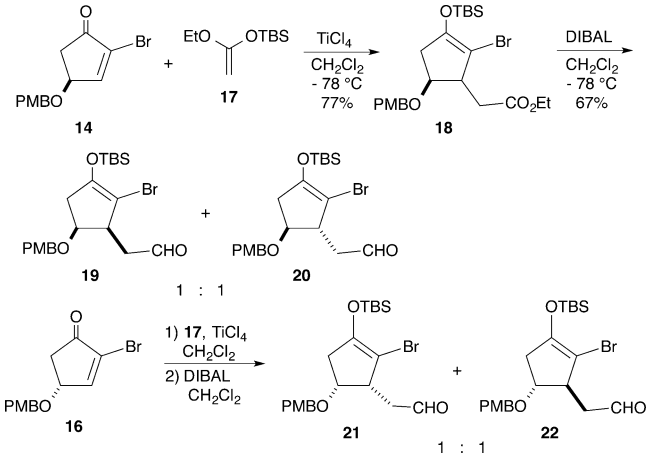
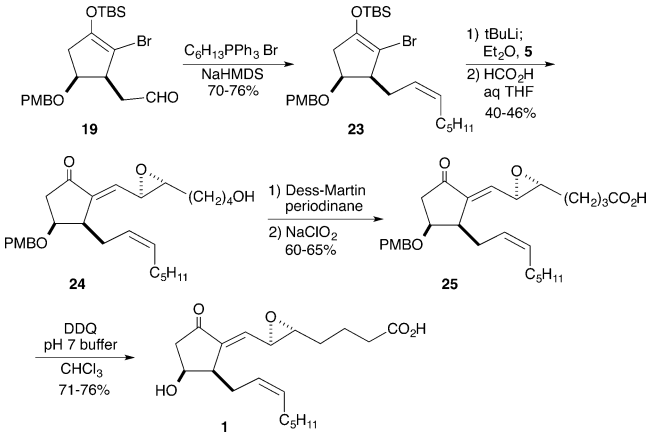
Scheme 3. Synthesis of Aldehydes 19−22



ethers **19** and **20**. Starting with the enantiomeric enone **16**, an identical series of reactions (silyl transfer and reduction) gave the two separable aldehydes **21** and **22** in similar yields.

With this protocol established, we were able to prepare sufficient quantities of the building blocks **19**, **20**, **21**, and **22** for completion of the synthesis. We then followed our previously developed triply convergent coupling strategy to afford the four EI analogues (Scheme 4).[13] Thus, we first

Scheme 4. Synthesis of EI Analogue 1



carried out an *E*-selective olefination via a Wittig reaction to convert the aldehyde, e.g., **19**, to the desired *Z*-alkene, e.g., **23**, in yields ranging from 70% to 76%. The coupling of the cyclopentenyl bromide with the epoxyaldehyde **5** (or **6**) was carried out by first forming the alkenyllithium species by treatment of the bromide with *tert*-butyllithium, followed by addition of the epoxyaldehyde to give the allylic alcohol. Treatment of this intermediate with aqueous formic acid effected hydrolysis of the silyl enol ether with concomitant dehydration and deprotection of the primary silyl ether to give the desired PMB-protected primary alcohol, e.g., **24**, a process that occurred in 40−46% yield. A two-step oxidation of the primary alcohol to the acid (Dess−Martin periodinane and then chlorite oxidation) furnished the PMB ether of EI, e.g., **25**, in yields of 60−65% for the two steps. Final oxidative cleavage of the PMB ether using dichlorodicyanoquinone (DDQ) in a mixed solvent system (chloroform and pH 7 buffer) afforded the desired EI analogues, e.g., **1**, in 71−76% yields. The analogous set of reactions on the other four aldehydes, **20**, **21**, and **22**, gave the expected EI analogues, **2**, **3**, and **4**.

Since the two trans enantiomers, **3** and **4**, showed very promising biological results (see later), we reexamined the synthetic route in an attempt to improve the selectivity for the trans isomer (Scheme 5). Our initial attempts to use a vinylcuprate reagent[19,20] for the 1,4-addition resulted in the formation of a 2:1 ratio of the desired trans isomer to the cis isomer. However, we also isolated up to 30% of an unexpected byproduct, namely, the compound in which the vinyl bromide had been replaced by a TBS group. Thus, treatment of **14** with vinylmagnesium bromide in the presence of copper iodide and TBSCl in THF/HMPA gave 44% of the desired trans compound **26** and 22% of the cis compound **27** along with 30% of the silylated silyl enol ether **28** (Table 1). Addition of lithium chloride gave no improvement, while changing the solvent to diethyl ether raised the ratio of trans to cis to 3:1 but still produced the unusual product. We reasoned that the byproduct was perhaps produced because of some free radical species formed during the preparation of the cuprate reagent. Therefore, we decided to add some styrene as a radical scavenger prior to the addition of the enone and the trapping with TBSCl. As shown in Table 1, by increasing the amounts of the additive styrene, we were able to completely prevent the formation of the byproduct **28**. Thus, the use of 2 equiv of styrene produced an inseparable 4:1 mixture of the trans and cis disubstituted bromoenol ethers **26** and **27** with none of the unusual product **28** observed. Although these compounds could not be separated at this stage, the corresponding aldehydes, **19** and **20**, could be easily separated, namely, after hydroboration−oxidation of the vinyl group followed by Dess−Martin periodinane oxidation of the resulting alcohol. By using the optimized conditions were we able to prepare the desired trans aldehyde **20** from **14** in 63% yield over three steps. By use of this and our previously established synthetic route, the two trans enantiomers of EI, **3** and **4**, were synthesized in reasonable quantities from **20** and **22** using the epoxyaldehydes **6** and **5**, respectively (**20** and **6** gave **3**, and **22** and **5** gave **4**). Finally PEIPC4 was prepared by the method described in detail in our earlier paper,[8] namely, the coupling of lyso-PC with the PMB ether of EI4 followed by removal of the PMB ether with DDQ.

Comparison of the Biological Effects of EI Stereoisomers. With these EI analogues in hand, we used qPCR to examine their activity in regulating genes from several pathways previously identified as regulated in HAEC by Ox-PAPC.[7] Treatment of HAECs with 3 μM EI1, EI2, EI3, or EI4 induced expression of IL-8, HO-1, ATF-3, and HSP1A1, representative genes from the inflammatory, oxidative stress, unfolded protein response, and stress response pathways (Figure 2). However, EI3 and EI4 induced these genes to a greater extent. Furthermore, EI4 induced these genes to a slightly greater extent than EI3 over the course of several experiments.

Comparison of the Effect of EI3 and EI4 with the Effect of PEIPC4. Since the fatty acid EI4 appeared to be most active on the regulation of these important hub genes (Figure 2), we synthesized the PEIPC isomer with EI4 in the *sn*-2 position of the molecule (PEIPC4) (Figure 3A). Using qPCR, we then compared the ability of EI3/4 with the effects of PEIPC4 in regulating four important genes (Figure 3B). Since the fatty acid EI4 appeared to be most active on the regulation of these important hub genes (Figure 2), we synthesized the PEIPC isomer with EI4 in the *sn*-2 position of the molecule

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Scheme 5. Improved Synthesis of Aldehyde 20

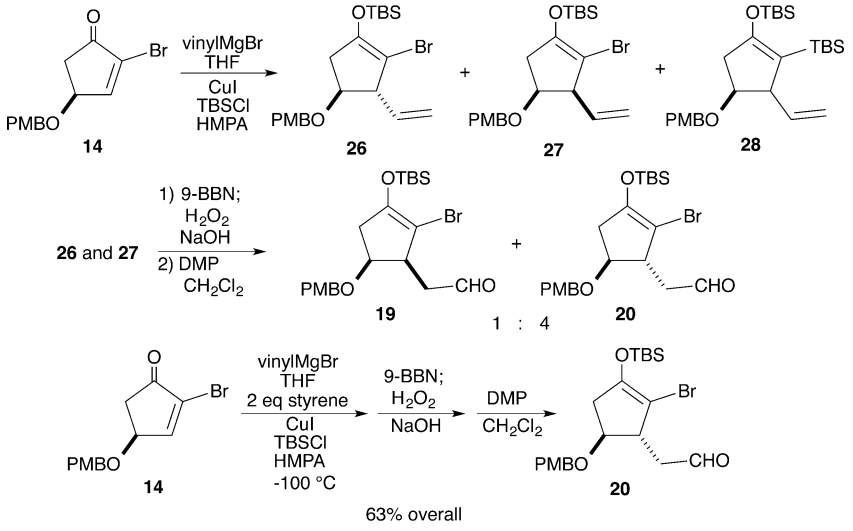
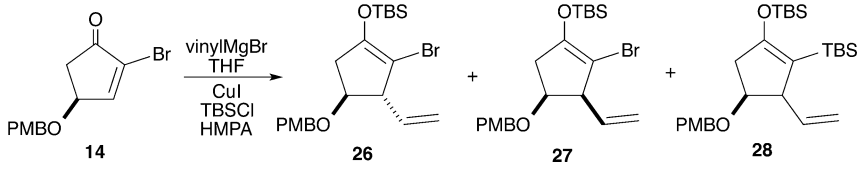


Table 1. 1,4-Addition of Vinylmagnesium Bromide to Enone 14



entry	additive	solvent	temp (°C)	26/27/28
1	none	THF	−78	44:22:30
2	LiCl	THF	−78	40:21:28
3	none	Et_2O	−100	48:16:30
4	1 equiv of styrene	Et_2O	−100	64:16:15
5	1.5 equiv of styrene	Et_2O	−100	68:17:6
6	2 equiv of styrene	Et_2O	−100	72:18:0

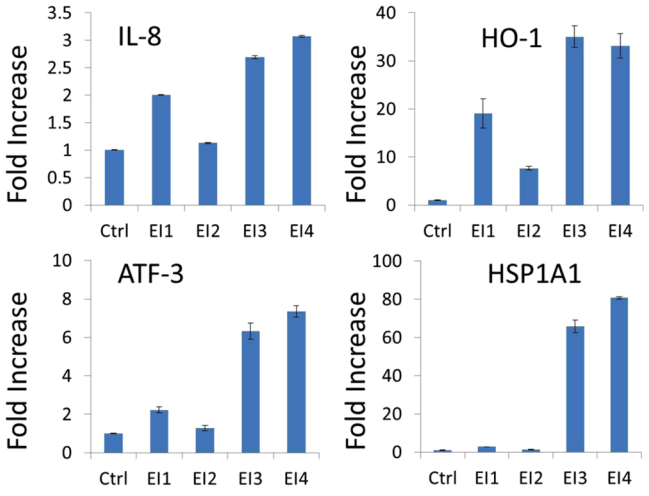


Figure 2. Comparison of the effect of the four EI isomers on gene regulation in HAECs. Duplicate wells of cultured HAECs were treated for 4 h in medium containing 1% FBS with or without 3 μM EI isomer **1**, **2**, **3**, or **4**. Cells were scraped, and IL-8, HO-1, ATF-3, and MCP-1 mRNA levels were measured with qPCR. GAPDH was used to normalize mRNA levels, and fold changes between untreated and treated cells were obtained. Representative experiment with three replicate wells is shown.

(PEIPC4) and compared its activity with EI3 and EI4. The PEIPC isomer **4** was prepared by the coupling of the components **22** and **5** by the method we have already described.[13] HAECs were treated for 4 h with EI3, EI4, and PEIPC4, and qPCR was performed. HO-1 and ATF-3 were induced by all three lipids though these were more strongly regulated by EI3/4 (Figure 3B). The most striking difference was seen in the effects of EI3/4 and PEIPC4 on MCP-1 gene regulation. Both EI3 and EI4 strongly reduced MCP-1 mRNA levels, while PEIPC4 strongly up-regulated MCP-1. We also tested the effect of EI4 on the protein levels of IL-8 and MCP-1, both of which have been shown to play a role in atherosclerosis. HAECs were treated for 4 h with varying levels of EI4, medium was collected, and levels of protein were measured by ELISA (Figure 4). We observed that IL-8 protein was modestly increased at low levels of EI and actually decreased at higher EI concentrations. MCP-1 protein was strongly decreased as had been shown for MCP-1 message.

Treatment of HAEC with EI Results in a Lower Induction of Inflammatory Genes than Treatment with PEIPC. We performed microarray analysis comparing the genes regulated by EI4 to the genes regulated by mixed isomers of PEIPC (Figure 5 and Supporting Information Table 1). Mixed isomers of PEIPC obtained from Ox-PAPC (subsequently referred to as PEIPC) were employed in this array analysis so that data could be compared to our previously published studies using mixed isomers. There was an approximately 50%

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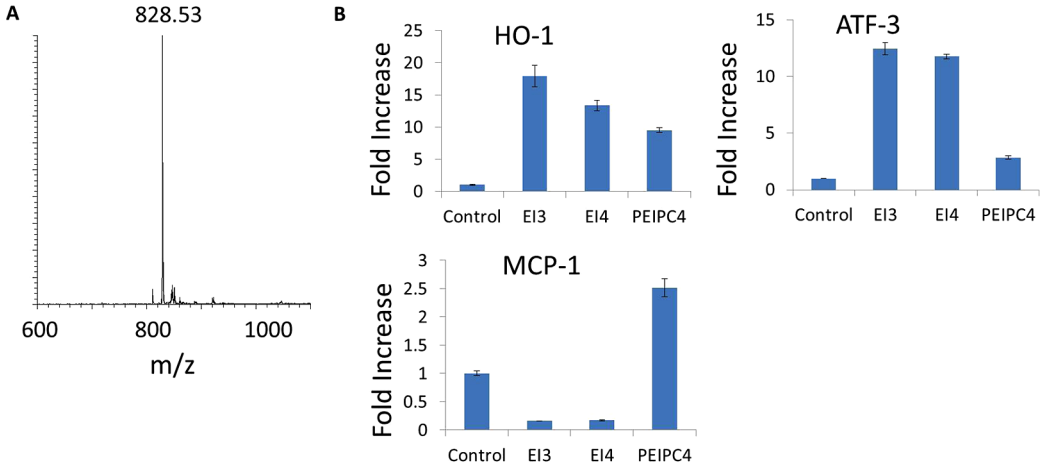

Figure 3. Comparison of gene regulation by EI4 present in a phospholipid (PEIPC4) and as a free fatty acid (EI4). (A) Full MS spectra of synthesized PEIPC4 (i.e., PEIPC containing EI isomer 4). (B) Duplicate wells of HAECs were treated for 4 h without or with EI3, EI4, or PEIPC4. Cells were collected and selected gene expression was measured by PCR as described for Figure 2. Representative experiment with three replicate wells is shown.

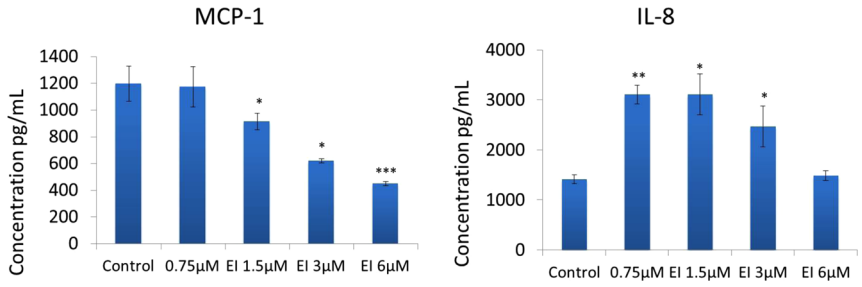

Figure 4. Dose–response effect of EI treatment on IL-8 and MCP-1 protein. HAEC triplicate wells were treated for 4 h with or without the indicated doses of EI. Medium was collected, and concentrations were measured. Student *t* test was performed for significance in difference with control samples with (∗) $p < 0.05$, (∗∗) $p < 0.01$, and (∗∗∗) $p < 0.001$.

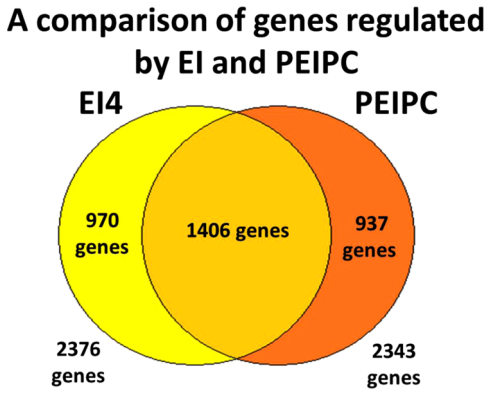

Figure 5. Microarray analysis: Venn diagram of genes regulated by 6 μM PEIPC vs 3 μM EI4 following a 4 h treatment. Data are filtered for $p \leq 0.001$ and fold change of 1.2 by lipids compared to untreated cells.

Table 2. Inflammatory Genes Regulated by EI[a]

gene	FC EI	FC PEIPC
MCP-1	0.2	13
CXCL1	1.3	4.8
NfKB1A	0.79	4.0
CXCL6	0.59	1.4
IL-8	3.0	17
VCAM-1	0.27	1.2
E-selectin	0.21	2.38[b]
ICAM-1	0.75	2.3
IL-6	0.6	2.2
RelB	0.8	4.2

[a]The microarrays shown in Figure 5 were used to examine the effect of EI and PEIPC on inflammatory genes most correlated with MCP-1 levels. [b]E-selectin levels from PEIPC4 qPCR results.

overlap in genes regulated by PEIPC and EI4. Both lipids similarly regulated genes associated with oxidative stress, UPR, and cell migration. However, there was a difference in the regulation of inflammatory genes by PEIPC and EI4 (Table 2). Compared to untreated cells, treatment of cells for 4 h with PEIPC increased expression of 10 major inflammatory genes, while EI either inhibited or only minimally increased expression of these genes.

EI4 Inhibits the Inflammatory Effects of IL-1β. On the basis of the effects of EI4 on inflammatory molecules, we hypothesized that EI4 might inhibit the effect of a major proinflammatory cytokine, IL-1β, which accumulates in atherosclerotic lesions. The data obtained indicate that exposure to 3 μM EI4 for 1 h followed by co-treatment with EI4 and IL-1β for 4 h led to a strong inhibition of the IL-1β increase in MCP-1 mRNA (Figure 6A). Levels of MCP-1 protein were also strongly decreased by pre- and co-treatment with EI4 (Figure 6B). Message levels of a group of proinflammatory genes induced by IL-1β were also decreased by pretreatment with EI4 (Table 3).

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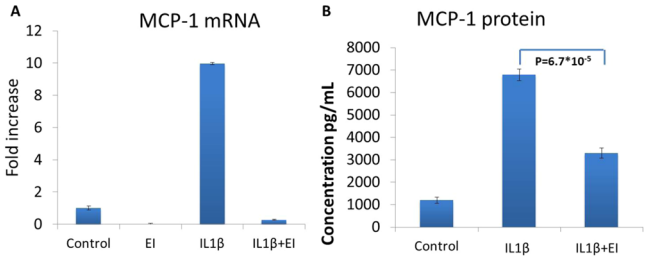

Figure 6. (A) MCP-1 mRNA regulation by IL-1β in the presence or absence of EI. HAECs were treated for 1 h with or without 3 μM EI and then for 4 h with or without IL-1β in the presence of EI. Cells were collected and PCR performed. (B) Levels of MCP-1 protein in medium of cells treated as shown in (A). (A) shows a representative experiment of three replicate wells. In (B), IL1β and IL1β + EI showed a significant difference in protein value, with p value as indicated.

Table 3. Proinflammatory Genes Induced by IL-1β and Induction Reduced by EI[a]

gene name	IL1β fold change	% reduction by EI
MCP-1	25.98 ± 1.97	79.6
ICAM-1	41.21 ± 8.89	52.3
VCAM-1	259.21 ± 79.16	92.1
E-selectin	205.03 ± 29.24	69.5
IL-6	206.05 ± 15.31	76.0
CXCL1	121.97 ± 4.90	53.4
CXCL6	86.43 ± 2.35	87.6

[a]Cells were treated as for Figure 6, and PCR was performed.

DISCUSSION

Studies from our group suggested the importance of the EI group in the action of Ox-PAPC and PEIPC.[9−11] Thus, we reasoned that EI might have a similar effect to PEIPC and would be a molecule that was easier to synthesize, more stable, and easier to work with that would allow us to easily compare the effects of the stereoisomers of the active group. We therefore developed a rapid method for the synthesis of EI. This method features lanthanide-promoted PMB ether formation, enzymatic kinetic resolution of the mono-PMB protected cyclopent-1-ene-3,5-diol, a $TiCl_4$ catalyzed silyl transfer of a silyl ketene acetal, and the 1,4-addition of a vinylcuprate reagent using styrene as a radical scavenger to avoid the formation of an unusual byproduct. We had previously shown that there were at least four regioisomers of PEIPC in Ox-PAPC and that the 5,6-epoxide isomer was the most active.[9] Therefore, in these studies we developed a simple method to produce several stereoisomers of EI with the epoxide group in the 5,6-position. Isomers **1** and **2** had much less activity than **3** and **4** (Figure 2), suggesting considerable specificity in the interaction of EI with the cell surface. It seems like the trans arrangement of the hydroxyl and the adjacent 2*Z*-octenyl chains is required for high activity, since the cis isomers show much lower activity. However, we have no good explanation for why that is the case except that that arrangement must allow the trans isomers to interact better with their biological target.

We showed differences in the effect of EI4 and PEIPC4 using qPCR (Figure 3). To gain a more in depth comparison between the effects of EI4 and PEIPC, we performed microarray analysis (Figure 5 and Supporting Information Table 1). Although EI contained the most active part of PEIPC, there was only a 50% overlap between PEIPC and EI in genes regulated by at least 1.2-fold in HAECs. Protective pathways, including UPR and oxidative stress, were regulated by both molecules, suggesting that EI will be useful in identifying these common pathways. However, there were differences in the effects of EI4 and PEIPC on some inflammatory molecules. In contrast to our published PEIPC data which showed a positive linear dose−response on IL-8 and MCP-1 protein,[9] EI actually dose dependently decreased the constitutive levels of MCP-1 and showed a bell shaped curve and a relatively low induction of IL-8. Compared to PEIPC, EI also showed a lower increase in message levels of eight other inflammatory molecules (Table 2). The difference in induction by EI and PEIPC was less for some molecules such as IL-6, and other inflammatory molecules were equally affected by both lipids (data not shown). Importantly, we have shown for the first time that IL-1β induction of a number of inflammatory mediators was also inhibited by EI (Table 3). IL-1β is a cytokine that accumulates in mice atherosclerotic lesions, and knockout of IL-1β in mice decreases atherosclerosis.[21] Furthermore, levels of IL-1β in human plasma are increased in atherosclerosis and are elevated in human lesions.[22−24] Two clinical trials using an inhibitor of IL-1β action have been initiated.

The data on effects of PEIPC and EI on inflammatory molecules in HAEC would suggest that PEIPC is more proinflammatory. In HAEC, in specific experimental conditions, this does appear to be the case. However, even in HAEC, PEIPC at low concentrations decreases breakdown of the endothelial barrier (increased breakdown is seen in inflammation), whereas at higher concentrations PEIPC stimulates barrier breakdown.[25] In dendritic cells PEIPC strongly inhibits the proinflammatory action of bacterial lipids.[26,27] While the effect of PEIPC is more complex, the effect of EI is mainly anti-inflammatory in both HAEC and dendritic cells. Taken together, these data suggest that both EI and PEIPC can be anti-inflammatory at some concentrations in some cell types. It is not yet known what determines the differences in effects on inflammatory molecules by the two lipids in different cell types. One possibility is that in dendritic cells PEIPC is more rapidly degraded to EI by phospholipases. In depth kinetic studies will be important to resolve these issues.

The phospholipase that might be responsible for EI release in vivo has not been determined. However, we previously showed that treatment of PEIPC with snake venom PLA_2 decreased the ability of PEIPC to induce monocyte binding.[11] At the time we interpreted this finding to suggest that EI was inactive. However, in light of the present findings, it is likely that EI actually inhibits monocyte binding. It has been shown that several mammalian PLA_2, Lp-PLA_2[28] and sPLA_2-5,[29] are increased in atherosclerotic lesions. Ox-PAPC treatment of endothelial cells and exposure to bacterial lipids in dendritic cells lead to increased levels of Lp-PLA_2 message.[7,26] Lp-PLA_2 has been shown to release F_2 prostaglandins from phospholipids[30] and may have the capacity to release isoprostanes, such as EI, from the phospholipid backbone. The ability of isoprostanes to be released from phospholipids is well documented and involves more than one PLA_2.[31] In preliminary experiments we have shown an increase in EI in Ox-PAPC treated HAEC perhaps caused by the increase in PLA_2's seen in these cells.

CONCLUSION

In summary, we have developed a new and simple method to synthesize four stereoisomers of EI and shown that isomer **4** is

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the most active in regulating endothelial cell function. Using this synthetic EI, we have demonstrated a major difference in the actions of the EI isoprostane and EI esterified to a phospholipid. We identified EI as a potent anti-inflammatory molecule in HAEC. During the preparation of this manuscript, Egger et al. published another method to prepare EI and evaluated the effects of EI (and mainly its dehydration product) in reducing secretion of proinflammatory cytokines IL-6 and IL-12 by dendritic cells in response to a TLR4 agonist.[27] Taken together, the data from Egger et al. and the data presented here on IL-1β suggest that EI is a regulator of both acute and chronic inflammation acting on two major inflammatory cell types, endothelial cells and dendritic cells. These data suggest that EI may be a useful anti-inflammatory agent.

EXPERIMENTAL SECTION

Biology. *Human Aortic Endothelial Cell Culture.* HAECs were isolated as described previously. HAECs were cultured in VEC complete medium (VEC technologies), and medium was changed to 10% FBS (Thermo Scientific) in M199 medium (Mediatech) overnight before use in experiments.

Measurement of EI and PEIPC Regulation of Genes Using PCR. HAECs were treated with 3 μM EI1-EI4 or 3 μM PEIPC **4** in M199 medium containing 1% FBS for 4 h. Cells were washed with PBS and lysed, RNA was extracted, and qPCR analysis was performed, measuring GAPDH, IL-8, HO-1, ATF-3, MCP-1, and/or HSP1A1 mRNA levels. GAPDH levels showed minimal change. IL-8, HO-1, ATF-3, MCP-1, and HSP1A1 levels were normalized to GAPDH levels. The primer sequences used for qPCR were the following: GAPDH, forward 5′-CCT CAA GAT CAT CAG CAA TGC CTC CT-3′, reverse 5′-GGT CAT GAG TCC TTC CAC GAT ACC AA-3′; HO-1, forward 5′-ATA GAT GTG GTA CAG GGA GGC CAT CA-3′, reverse 5′-GGC AGA GAA TGC TGA GTT CAT GAG GA-3′; IL-8, forward 5′-ACC ACA CTG CGC CAA CAC AGA AAT-3′, reverse 5′-TCC AGA CAG AGC TCT CTT CCA TCA GA-3′; ATF-3, forward 5′-TTG CAG AGC TAA GCA GTC GTG GTA-3′, reverse 5′-ATG GTT CTC TGC TGC TGG GAT TCT-3′; MCP-1, forward 5′-TGC TCA TAG CAG CCA CCT TCA TTC-3′, reverse 5′- GAC ACT TGC TGC TGG TGA TTC TTC; HSP1A1, forward 5′-AAC CAC TTC GTG GAG GAG TTC AAG-3′, reverse 5′-TAG AAG TCG ATG CCC TCA AAC AGG-3′.

Microarray Analysis. Duplicate wells of HAECs were treated with 1% M199 medium with or without 3 μM EI4 (or PEIPC4) for 4 h, and RNA was extracted. For microarray analysis, RNA was prepared for hybridization to Illumina arrays, measuring 45 000 probes, using a standard protocol described previously.[7] Data were analyzed using the Genome Studio software package. During data analysis, data were analyzed using background subtraction, quantile normalization, and the content descriptor file "HumanHT-12_V4_0_R1_15002873_B.bgx" in the Genome Studio files. The data were filtered for results with the detection p value less than 0.001 for the EI/PEIPC RNA measurement and for the control measurement. The fold changes in probes were calculated as the average probe level for each treatment divided by the average probe level for control treatment and converted to ln values to analyze for gene regulation. Up-regulated/down-regulated genes were identified as those that changed at least 1.2-fold from control values in the presence of lipids.

Cell Treatment for Analysis of EI4 Effects on IL-8 and MCP-1 Proteins and Effects of EI on IL-1β Induction of MCP-1 Message and Protein. For dose−response studies, HAECs were untreated or treated with increasing concentrations of EI for 4 h in 199 medium containing 1% FBS. Medium was collected, and levels of IL-8 and MCP-1 were determined using ELISA kits from RD Systems. To determine the effect of EI on IL-1β message and protein, cells were pretreated for 1 h with 3 μM EI4, then treated with 20 ng/mL IL-1β for 4 h. For qPCR, cells were collected as above and MCP-1 message was determined. For MCP-1 protein measurements, medium was collected and protein determined using ELISA.

Chemistry. *General.* All reactions were carried out under an argon atmosphere unless otherwise specified. Tetrahydrofuran (THF), diethyl ether, toluene, and benzene were distilled from benzoquinone ketyl radical under an argon atmosphere. Dichloromethane (DCM) and triethylamine (TEA) were distilled from calcium hydride under an argon atmosphere. All other solvents or reagents were purified according to literature procedures. ^{1}H NMR spectra were recorded at 200 and 400 MHz and are reported relative to deuterated solvent signals. Data for ^{1}H NMR spectra are reported as follows: chemical shift (δ ppm), multiplicity, coupling constant (Hz), and integration. Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. ^{13}C NMR spectra were recorded at 50 and 100 MHz. Data for ^{13}C NMR spectra are reported in terms of chemical shift. The chemical shifts are reported in parts per million (ppm, δ). Thin-layer chromatography (TLC) was carried out using precoated silica gel sheets. Visual detection was performed using ceric ammonium nitrate or p-anisaldehyde stains. Flash chromatography was performed using SilicaFlash P60 (60 Å, 40−63 μm) silica gel with compressed air. The purity of the compounds was assayed by high field proton and carbon NMR and was ≥95% except for mixtures of isomers (diastereomers) as pointed out in the text.

(RS)-4-(4-Methoxyphenylmethoxy)cyclopent-2-enone, ***9****.* To a solution of 4-hydroxycyclopentenone **8** (13 g, 132.5 mmol) and freshly prepared 4-methoxybenzyl trichloroacetimidate (**19**) (55.3 g, 195.7 mmol) in toluene (1 L) was added lanthanide tris-(trifluoromethanesulfonate) (3.88 g, 6.62 mmol) at 21 °C. The reaction mixture was stirred for 5 min and then concentrated to dryness at 40 °C in vacuo. The residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 3:1, v/v) to afford the desired product **9** (26.1 g, 119.6 mmol, 90%) as a colorless oil: R_f = 0.48 (hexane/ethyl acetate, 2:1, v/v); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.52 (dd, J = 5.7, 2.3 Hz, 1H), 7.19 (d, J = 8.6 Hz, 2H), 6.81 (d, J = 8.8 Hz, 2H), 6.18 (dd, J = 5.8, 1.2 Hz, 1H), 4.70−4.64 (m, 1H), 4.50 (d, J = 11.2 Hz, 1H), 4.47 (d, J = 11.2 Hz, 1H), 3.73 (s, 3H), 2.59 (dd, J = 18.4, 6.0 Hz, 1H), 2.26 (dd, J = 18.4, 2.2 Hz, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 205.9, 161.1, 159.3, 135.4, 129.4, 113.8 (3 C's), 76.4, 71.4, 55.1, 41.6; ESI-HRMS found 241.0838 $[M + Na]^+$, calcd for $C_{13}H_{14}O_3Na$ 241.0841.

(±)-(1S,4R)-4-(4-Methoxyphenylmethoxy)cyclopent-2-enol, ***10****.* To a solution of (*RS*)-4-(4-methoxyphenylmethoxy)cyclopent-2-enone, **9** (14.8 g, 67.8 mmol), and cerous chloride heptahydrate (27.8 g, 74.6 mmol) in methanol (180 mL) was added sodium borohydride (1.92 g, 50.8 mmol) portionwise at −78 °C. The reaction mixture was stirred for 1 h and then concentrated to dryness in vacuo. The residue was diluted with ethyl acetate (500 mL) and then washed with aqueous saturated ammonium chloride solution (500 mL) and brine (500 mL). The organic layer was dried over $MgSO_4$, filtered, and then concentrated to dryness in vacuo. The residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 2:1, v/v) to afford the desired product **10** (14.0 g, 63.6 mmol, 94%) as a colorless oil: R_f = 0.25 (hexane/ethyl acetate, 2:1, v/v); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.18 (d, J = 8.6 Hz, 2H), 6.78 (d, J = 8.6 Hz, 2H), 5.89 (bs, 2H), 4.49−4.46 (bm, 1H), 4.36 (d, J = 11.4 Hz, 1H), 4.33 (dd, J = 7.0, 4.4 Hz, 1H), 4.32 (d, J = 11.4 Hz, 1H), 3.71 (s, 3H), 3.42−3.39 (bm, 1H), 2.55 (ddd, J = 14.0, 7.2, 7.2 Hz, 1H), 1.54 (ddd, J = 14.0, 4.4, 4.4 Hz, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 158.9, 137.0, 133.4, 130.1, 129.2, 113.5, 81.0, 74.3, 70.3, 54.9, 40.5; ESI-HRMS found 243.0989 $[M + Na]^+$, calcd for $C_{13}H_{16}O_3Na$ 243.0997.

(1R,4S)-4-(4-Methoxyphenylmethoxy)cyclopent-2-enol, ***11****, and (1S,4R)-1-Acetyloxy-4-(4-methoxyphenylmethoxy)cyclopent-2-ene,* ***12****.* To a solution of racemic (1*S*,4*R*)-4-(4-methoxyphenylmethoxy)-cyclopent-2-enol, **10** (11.8 g, 53.6 mmol) in methyl ethyl ketone (2.5 L) were added vinyl acetate (450 mL), water (2.5 mL), and lipase AK (Amano Enzyme U.S.A. Co., Ltd., 35 g). The reaction mixture was stirred at 21 °C for 20 h and then filtered. The enzyme cake was washed with methyl ethyl ketone (3 × 50 mL) and then air-dried for future use. The filtrates were concentrated to dryness in vacuo. The residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 5:1, v/v) to afford the desired products **11**

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(7.13 g, 25.6 mmol, 48%, 97% ee) and **12** (5.58 g, 25.3 mmol, 47%, 99% ee) as colorless oils.

Compound **11**: The NMR and MS data were the same as for the racemic compound **10**.

Compound **12**: R_f = 0.70 (hexane/ethyl acetate, 2:1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.25 (d, J = 8.7 Hz, 2H), 6.85 (d, J = 8.7 Hz, 2H), 6.09 (ddd, J = 5.6, 1.6, 1.3 Hz, 1H), 5.97 (ddd, J = 5.6, 1.7, 1.4 Hz, 1H), 5.48 (m, 1H), 4.50 (d, J = 11.4 Hz, 1H), 4.46 (d, J = 11.4 Hz, 1H), 4.50−4.44 (m, 1H), 3.78 (s, 3H), 2.75 (dd, J = 14.2, 7.3, 7.3 Hz, 1H), 2.03 (s, 3H), 1.75 (ddd, J = 14.2, 4.4, 4.4 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.8, 159.2, 136.2, 132.7, 130.4, 129.3, 113.8, 80.9, 70.7, 55.2, 37.7, 37.6, 21.1; ESI-HRMS found 301.1044 [M + Na]$^+$, calcd for $C_{15}H_{18}O_4Na$ 301.1052.

To a solution of compound **11** (163 mg, 0.74 mmol) in pyridine (3 mL) were added acetic anhydride (0.3 mL) and 4-(dimethylamino)-pyridine (DMAP, 5 mg). The reaction mixture was stirred at 21 °C overnight and then diluted with diethyl ether (20 mL). The resulting organic layer was washed with 1 N aqueous HCl (3 × 20 mL), saturated $NaHCO_3$ (20 mL), and brine (20 mL). The organic layer was dried over $MgSO_4$, filtered, and then concentrated to dryness in vacuo. The residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 5:1, v/v) to afford the desired product (201 mg, 0.72 mmol, 97%) as a colorless oil. The NMR and MS data were the same as those for compound **12**. The enantiomeric excess was determined by chiral GC.

(1S,4R)-4-(4-Methoxyphenylmethoxy)cyclopent-2-enol. To a solution of 1-acetyloxy-4-(4-methoxyphenylmethoxy)cyclopent-2-ene, **12** (30 g, 0.108 mol), in methanol (200 mL) was added potassium carbonate (16.39 g, 0.119 mol). The reaction mixture was stirred at 21 °C overnight and then concentrated at to dryness in vacuo. The residue was diluted with dichloromethane (500 mL) and then washed with 5% aqueous HCl (500 mL) and brine (500 mL). The organic layer was dried over $MgSO_4$, filtered, and then concentrated to dryness in vacuo. The residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 2:1, v/v) to afford the (1*S*,4*R*) 4-(4-methoxyphenylmethoxy)cyclopent-2-enol (23.3 g, 0.106 mol, 98%) as a colorless oil. The NMR and MS data were the same as the compound **10**.

*General Procedure for the Preparation of Compounds **13** and **15**.* To a solution of 4-(4-methoxyphenylmethoxy)cyclopent-2-enol (5.51 g, 25.0 mmol) in dichloromethane (50 mL) was added a mixture of pyridinium chlorochromate (7.55 g, 35.0 mmol) and Celite (7.55 g) portionwise at 0 °C. The reaction mixture was stirred overnight at 21 °C. Isopropyl alcohol (60 mL) was then added and the mixture stirred for 30 min. The solution was filtered through a pad of Celite and silica gel (4 cm), washed with ethyl acetate (2 × 60 mL), and then concentrated to dryness in vacuo. The residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 2:1, v/v) to afford the 4-(4-methoxyphenylmethoxy)cyclopent-2-enone product **13** or **15** (4.85 g, 22.2 mmol, 89%) as a colorless oil.

*(S)-4-(4-Methoxyphenylmethoxy)cyclopent-2-enone, **13**.* This compound was synthesized according to the general procedure for the preparation of compounds **13** and **15**. The NMR and MS data were the same as those for compound **9**.

*(R)-4-(4-Methoxyphenylmethoxy)cyclopent-2-enone, **15**.* This compound was synthesized according to the general procedure for the preparation of compound **13** and **15**. The NMR and MS data were the same as those for compound **9**.

*General Procedure for the Preparation of Compounds **14** and **16**.* To a solution of 4-(4-methoxyphenylmethoxy)cyclopent-2-enone (20.5 g, 93.9 mmol) in dichloromethane (240 mL) was added bromine (4.81 mL, 93.9 mmol) dropwise at 0 °C. The reaction mixture was stirred for 5 min, and then triethylamine (17 mL, 122 mmol) was added. The resulting mixture was stirred for 30 min at 21 °C and then poured into water (200 mL). The aqueous layer was extracted with dichloromethane (3 × 200 mL). The combined organic layers were dried over $MgSO_4$, filtered, and then concentrated to dryness in vacuo. The residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 4:1, v/v) to afford the 2-bromo-4-(4-methoxyphenylmethoxy)cyclopent-2-enone product (22.3 g, 75.1 mmol, 80%) as a pale yellow oil.

*1(S)-2-Bromo-4-(4-methoxyphenylmethoxy)cyclopent-2-enone, **14**.* This compound was synthesized according to the general procedure for the preparation of compound **14** and **16**. R_f = 0.60 (hexane/ethyl acetate, 2:1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.67 (d, J = 2.4 Hz, 1H), 7.26 (d, J = 8.7 Hz, 2H), 6.90 (d, J = 8.7 Hz, 2H), 4.67 (ddd, J = 8.0, 2.6, 2.4 Hz, 1H), 4.58 (d, J = 11.3 Hz, 1H), 4.55 (d, J = 11.3 Hz, 1H), 3.86 (s, 3H), 2.82 (dd, J = 18.4, 6.0 Hz, 1H), 2.47 (dd, J = 18.4, 2.0 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 197.8, 159.7, 158.7, 129.6, 129.1, 128.9, 114.1, 75.0, 71.6, 55.3, 40.6; ESI-HRMS found 318.9942 [M + Na]$^+$, calcd for $C_{13}H_{13}BrO_3Na$ 318.9946.

*(R)-2-Bromo-4-(4-methoxyphenylmethoxy)cyclopent-2-enone, **16**.* This compound was synthesized according to the general procedure for the preparation of compounds **14** and **16**. The NMR and MS data were the same as those for compound **14**.

*General Procedure for the Preparation of Compound **18** (and Its Enantiomer) by the Titanium Tetrachloride Catalyzed Silyl Transfer Reaction.* To a solution of 2-bromo-4-(4-methoxyphenylmethoxy)-cyclopent-2-enone **14** (or **16**) (5.63 g, 18.9 mmol) in dichloromethane (280 mL) was added 1 M $TiCl_4$ in dichloromethane (1.89 mL, 1.89 mmol) dropwise at −78 °C. The resulting mixture was stirred at −78 °C for 30 min, and then freshly prepared ethyl (*tert*-butyldimethylsilyl)ketene acetal **17** (9.1 g, 45.0 mmol) was added dropwise over 30 min. The reaction mixture was stirred at −78 °C for 1 h and then quenched with triethylamine (4 mL). The solution was slowly warmed to 21 °C and then concentrated to dryness in vacuo. The residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 10:1, v/v) to afford the desired product **18** (7.7 g, 15.7 mmol, 83%) as a pale yellow oil.

*(1RS,5S)-Ethyl 2-Bromo-3-[(1,1-dimethylethyl)dimethylsilyloxy]-5-(4-methoxyphenylmethoxy)cyclopent-2-ene-1-acetate, **18**.* R_f = 0.48 (hexane/ethyl acetate, 10:1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.23 (m, 4H), 6.85 (m, 4H), 4.48−4.36 (m, 6H), 4.49 (d, J = 11.3 Hz, 1H), 4.44 (d, J = 11.3 Hz, 1H), 4.39 (d, J = 11.3 Hz, 1H), 4.35 (d, J = 11.3 Hz, 1H), 4.29 (ddd, J = 7.2, 5.2, 5.2 Hz, 1H), 4.14−4.04 (m, 5H), 3.79 (s, 6H), 2.70−2.20 (m, 8H), 1.26 (t, J = 7.2 Hz, 3H), 1.19 (t, J = 7.2 Hz, 3H), 0.95 (s, 9H), 0.948 (s, 9H), 0.20−0.15 (m, 12H); ESI-HRMS found 521.1317 [M + Na]$^+$, calcd for $C_{23}H_{35}BrO_5SiNa$ 521.1335.

*Enantiomer of **18**.* This compound was synthesized according to the general procedure for the preparation of compound **18**. The NMR and MS data were the same as those for compound **18**.

*General Procedure for the Preparation of 2-[2-Bromo-3-[(1,1-dimethylethyl)dimethylsilyloxy]-5-(4-methoxyphenylmethoxy)-cyclopent-2-ene]acetaldehyde **19**, **20**, **21**, and **22**. Method A.* To a solution of the starting material **18** (or its enantiomer) (7.7 g, 15.7 mmol) in dichloromethane (260 mL) was added 1 M DIBAL in dichloromethane (16.5 mL, 16.5 mmol) dropwise at −78 °C. The reaction mixture was stirred at −78 °C for 15 min and then quenched with saturated aqueous Rochelle's salt (260 mL). The solution was slowly warmed to 21 °C and stirred for 1 h. The organic layer was separated and extracted with dichloromethane (3 × 200 mL). The combined organic layers were dried over Na_2SO_4, filtered, and then concentrated to dryness in vacuo. The residue was purified by flash column chromatography over silica gel (pentane/diethyl ether, 20:1, v/v) to afford first the cis aldehyde **19** or **21** (2.92 g, 6.4 mmol, 41%) and then the trans aldehyde **20** or **22** (2.78 g, 6.1 mmol, 39%) as pale yellow oils.

Method B. To a solution of CuI (13.34 g, 70 mmol) in diethyl ether (400 mL) at −78 °C was added dropwise 1 M vinylmagnesium bromide in THF (140 mL). The reaction mixture was stirred at −20 °C for 20 min and then cooled to −78 °C. Freshly distilled styrene (8.03 mL, 70 mmol dried over CaH_2) was added and stirred at −78 °C for 20 min. The reaction mixture was cooled to −100 °C. A solution of 2-bromo-4-(4-methoxyphenylmethoxy)cyclopent-2-enone **14** or **16** (10.4 g, 35 mmol) in diethyl ether (70 mL) was added dropwise and stirred at −100 °C for 1 h. TBSCl (21.1 g, 140 mmol) in diethyl ether (35 mL) and HMPA (98.6 mL, 567 mmol) were subsequently added.

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The reaction mixture was allowed to warm to 21 °C and stirred overnight. The solution was quenched with a pH 7 buffer (500 mL), filtered through a pad of Celite, and then washed with diethyl ether (3 × 100 mL). The organic layer was washed with water (500 mL) and brine (500 mL). The organic layer was dried over Na_2SO_4, filtered, and then concentrated to dryness in vacuo. The residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 10:1, v/v) to afford a separable trans product (28.9 g, 65.8 mmol, 94%) as a pale yellow oil. R_f = 0.68 (hexane/ethyl acetate, 6:1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.26 (d, J = 8.8 Hz, 2H), 6.89 (d, J = 8.8 Hz, 2H), 5.69 (ddd, J = 18.6, 10.0, 8.4 Hz, 1H), 5.23 (d, J = 18.6 Hz, 1H), 5.19 (d, J = 10.0 Hz, 1H), 4.53 (d, J = 11.4 Hz, 1H), 4.45 (d, J = 11.4 Hz, 1H), 3.97 (ddd, J = 7.4, 4.0, 4.0 Hz, 1H), 3.81 (s, 3H), 3.38 (m, 1H), 2.61 (dd, J = 15.9, 7.6 Hz, 1H), 2.36 (ddd, J = 15.9, 4.0, 1.2 Hz, 1H), 0.97 (s, 9H), 0.21 (s, 3H), 0.20 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 159.3, 148.5, 138.6, 130.1, 129.3, 117.1, 113.8, 96.8, 80.1, 70.7, 57.0, 55.3, 39.3, 25.6, 18.1, −3.9; ESI-HRMS found 461.1116 [M + Na]$^+$, calcd for $C_{21}H_{31}BrO_3SiNa$ 461.1124. No undesired byproduct was formed. R_f = 0.70 (hexane/ethyl acetate, 6:1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.25 (d, J = 8.4 Hz, 2H), 6.88 (d, J = 8.4 Hz, 2H), 5.68 (ddd, J = 18.3, 10.0, 8.0 Hz, 1H), 5.05 (dd, J = 18.3, 1.5 Hz, 1H), 5.95 (dd, J = 10.0, 1.5 Hz, 1H), 4.68 (s, 1H), 4.53 (d, J = 11.6 Hz, 1H), 4.42 (d, J = 11.6 Hz, 1H), 3.80 (s, 3H), 3.82−3.70 (m, 1H), 3.38 (bd, J = 8.4 Hz, 1H), 2.71 (ddd, J = 16.5, 6.3, 0.6 Hz, 1H), 2.32 (dd, J = 16.5, 2.0 Hz, 1H), 0.94 (s, 9H), 0.89 (s, 9H), 0.16 (s, 3H), 0.12 (s, 3H), 0.09 (s, 3H), 0.01 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 161.7, 159.1, 141.7, 130.9, 129.1, 127.5, 114.2, 113.7, 113.6, 109.7, 83.1, 70.2, 64.7, 56.6, 55.3, 41.5, 27.3, 26.0, 18.4, −3.1, −3.2, −4.3, −4.5, −5.2; ESI-HRMS found 497.2878 [M + Na]$^+$, calcd for $C_{27}H_{46}O_3Si_2Na$ 497.2883.

To the vinyl bromide (9.95 g, 22.6 mmol) was added dropwise 0.5 M 9-BBN in THF (90.4 mL, 45.2 mmol) at 0 °C. The reaction mixture was then allowed to warm to 21 °C and stirred for 1.5 h. The solution was cooled to −78 °C, and a mixture of H_2O_2 (15 mL) and 3 N aqueous NaOH (15 mL) was added slowly. The reaction mixture was stirred for 1 h at 21 °C and then quenched with a pH 7 buffer (1 L). The aqueous layer was extracted with diethyl ether (3 × 500 mL). The combined organic layers were washed with brine (500 mL), dried over Na_2SO_4, filtered, and then concentrated to dryness in vacuo. The residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 3:1, v/v) to afford an inseparable mixture of the desired cis and trans alcohol products (9.5 g, 20.8 mmol, 92%) as pale yellow oils. R_f = 0.35 (hexane/ethyl acetate, 3:1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.26 (d, J = 8.8 Hz, 2H), 6.88 (d, J = 8.8 Hz, 2H), 4.48 (d, J = 11.2 Hz, 1H), 4.40 (d, J = 11.2 Hz, 1H), 3.97 (ddd, J = 7.2, 5.2, 5.2 Hz, 1H), 3.80 (s, 3H), 3.70 (t, J = 6.1 Hz, 1H), 2.81 (m, 1H), 2.60 (ddd, J = 15.2, 7.4, 1.8 Hz, 1H), 2.37 (ddd, J = 15.2, 5.2, 1.6 Hz, 1H), 2.07 (m, 1H), 1.46 (m, 1H), 0.96 (s, 9H), 0.20 (s, 3H), 0.19 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 159.5, 147.2, 129.6, 129.4, 128.6, 113.9, 98.5, 80.4, 70.8, 60.9, 55.3, 49.2, 38.9, 35.8, 27.4, 25.5, 22.7, 18.1, −3.9; ESI-HRMS found 479.1221 [M + Na]$^+$, calcd for $C_{21}H_{33}BrO_4SiNa$ 479.1229.

To a solution of the alcohol (4.19 g, 9.18 mmol) in dichloromethane (46 mL) at 0 °C was added solid $NaHCO_3$ (3.86 g, 45.9 mmol) followed by Dess−Martin periodinane (4.67 g, 11.0 mmol) portionwise. The reaction mixture was allowed to warm to 21 °C and stirred for 1 h. The solution was quenched with saturated aqueous $NaHCO_3$ (40 mL) and saturated aqueous $Na_2S_2O_3$ (40 mL). The aqueous layer was extracted with dichloromethane (3 × 50 mL). The combined organic layers were dried over Na_2SO_4, filtered, and then concentrated to dryness in vacuo. The residue was purified by flash column chromatography over silica gel (pentane/diethyl ether, 20:1, v/v) to afford first the cis aldehyde **19** or **21** (0.65 g, 1.43 mmol, 16%) and then the trans aldehyde **20** or **22** (2.68 g, 5.90 mmol, 64%) as pale yellow oils.

2-[(1S,5S)-2-Bromo-3-[(1,1-dimethylethyl)dimethylsilyloxy]-5-(4-methoxyphenylmethoxy)cyclopent-2-eneacetaldehyde ***19*** *and 2-[(1R,5R)-2-Bromo-3-[(1,1-dimethylethyl)dimethylsilyloxy]-5-(4-methoxyphenylmethoxy)cyclopent-2-eneacetaldehyde* ***21***. R_f = 0.42 (hexane/ethyl acetate, 4:1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.81 (dd, J = 1.4, 1.4 Hz, 1H), 7.18 (d, J = 8.7 Hz, 2H), 6.86 (d, J = 8.7 Hz, 2H), 4.40 (d, J = 11.2 Hz, 1H), 4.31 (d, J = 11.2 Hz, 1H), 4.28 (ddd, J = 12.3, 7.3, 4.9 Hz, 1H), 3.80 (s, 3H), 3.42 (m, 1H), 2.75 (ddd, J = 17.2, 9.4, 1.8 Hz, 1H), 2.55 (ddd, J = 17.2, 4.1, 1.1 Hz, 1H), 2.49 (ddd, J = 15.6, 7.2, 0.5 Hz, 1H), 2.36 (ddd, J = 15.6, 5.0, 2.0 Hz, 1H), 0.96 (s, 9H), 0.19 (s, 3H), 0.18 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 201.8, 159.3, 148.3, 129.9, 129.3, 113.8, 97.2, 74.9, 71.5, 55.3, 45.3, 41.9, 38.4, 25.5, 18.1, −3.9; ESI-HRMS found 477.1064 [M + Na]$^+$, calcd for $C_{21}H_{31}BrO_4SiNa$ 477.1073.

2-[(1S,5R)-2-Bromo-3-[(1,1-dimethylethyl)dimethylsilyloxy]-5-(4-methoxyphenylmethoxy)cyclopent-2-eneacetaldehyde ***20*** *and 2-[(1R,5S)-2-Bromo-3-[(1,1-dimethylethyl)dimethylsilyloxy]-5-(4-methoxyphenylmethoxy)cyclopent-2-eneacetaldehyde* ***22***. R_f = 0.38 (hexane/ethyl acetate, 4:1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.81 (dd, J = 2.4, 1.8 Hz, 1H), 7.25 (d, J = 8.7 Hz, 2H), 6.88 (d, J = 8.7 Hz, 2H), 4.46 (d, J = 11.9 Hz, 1H), 4.44 (d, J = 11.9 Hz, 1H), 3.86 (ddd, J = 7.5, 3.8, 3.8 Hz, 1H), 3.80 (s, 3H), 3.24 (m, 1 H), 2.73 (ddd, J = 16.5, 4.6, 1.7 Hz, 1H), 2.59 (ddd, J = 15.8, 7.3, 1.9 Hz, 1H), 2.41 (ddd, J = 16.5, 8.5, 2.6 Hz, 1H), 2.34 (ddd, J = 15.8, 4.0, 1.2 Hz, 1H), 0.96 (s, 9H), 0.19 (s, 3H), 0.18 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 201.5, 159.3, 148.6, 129.9, 129.4, 113.9, 96.4, 79.3, 70.8, 55.3, 47.4, 46.4, 39.2, 25.5, 18.1, −3.9; ESI-HRMS found 477.1062 [M + Na]$^+$, calcd for $C_{21}H_{31}BrO_4SiNa$ 477.1073.

General Procedure for the Preparation of 2-Bromo-3-[(1,1-dimethylethyl)dimethylsilyloxy]-4-(4-methoxyphenylmethoxy)-3-((Z)-oct-2-enyl)cyclopent-1-ene, ***23***. To a solution of hexyltriphenylphosphonium bromide (5.29 g, 12. Four mmol) in THF (24 mL) at 0 °C was added 1 M NaHMDS in THF (11.8 mL, 11.8 mmol). The reaction mixture was stirred at 0 °C for 1 h and then cooled to −78 °C. A solution of the aldehyde **19** (2.67 g, 5.9 mmol) in THF (12 mL) was added dropwise and then stirred at −78 °C for 1 h. The solution was quenched with a mixture of triethylamine, water, and diethyl ether (64 mL, 1:2:1, v/v/v) and then allowed to warm to 21 °C. The aqueous layer was extracted with diethyl ether (3 x50 mL). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4, filtered, and then concentrated to dryness in vacuo. The residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 6:1, v/v) to afford the desired *Z*-alkene, e.g., **23** (2.35 g, 4.48 mmol, 76%), as a pale yellow oil.

(3S,4S)-2-Bromo-1-[(1,1-dimethylethyl)dimethylsilyloxy]-4-(4-methoxyphenylmethoxy)-3-((Z)-oct-2-enyl)cyclopent-1-ene, ***23****, and Its (3R,4R)-Enantiomer.* R_f = 0.64 (hexane/ethyl acetate, 4:1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.25 (d, J = 8.4 Hz, 2H), 6.87 (d, J = 8.8 Hz, 2H), 5.52 (m, 1H), 5.41 (m, 1H), 4.46 (d, J = 11.2 Hz, 1H), 4.42 (d, J = 11.2 Hz, 1H), 4.27 (ddd, J = 7.2, 7.2, 7.2 Hz, 1H), 3.82 (s, 3H), 2.83 (bddd, J = 6.4, 6.4, 6.4 Hz, 1H), 2.42 (m, 4H), 2.07 (m, 2H), 1.35 (m, 6H), 1.01 (s, 9H), 0.91 (t, J = 6.8 Hz, 3H), 0.23 (s, 3H), 0.19 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 159.3, 147.8, 131.3, 130.4, 129.2, 129.1, 127.4, 113.8, 98.9, 71.4, 55.2, 49.3, 38.6, 31.7, 29.5, 27.5, 25.9, 25.6, 22.7, 18.1, 14.2, −3.9; ESI-HRMS found 545.2058 [M + Na]$^+$, calcd for $C_{27}H_{43}BrO_3SiNa$ 545.2063.

(3S,4R)-2-Bromo-3-[(1,1-dimethylethyl)dimethylsilyloxy]-4-(4-methoxyphenylmethoxy)-3-((Z)-oct-2-enyl)cyclopent-1-ene and Its (3R,4S)-Enantiomer. R_f = 0.61 (hexane/ethyl acetate, 4:1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.25 (d, J = 8.4 Hz, 2H), 6.87 (d, J = 8.8 Hz, 2H), 5.48 (m, 1H), 5.40 (m, 1H), 4.44 (d, J = 11.6 Hz, 1H), 4.40 (d, J = 11.6 Hz, 1H), 3.85 (ddd, J = 7.2, 2.8, 2.8 Hz, 1H), 3.80 (s, 3H), 2.80 (m, 1H), 2.56 (ddd, J = 16.0, 7.2, 2.0 Hz, 1H), 2.44 (ddd, J = 14.8, 7.6, 4.0 Hz, 1H), 2.31 (ddd, J = 16.0, 3.2, 0.7 Hz, 1H), 2.09 (ddd, J = 14.8, 7.2, 7.2 Hz, 1H), 2.06 (m, 2H), 1.36−1.27 (m, 6H), 1.04 (s, 9H), 0.95 (t, J = 6.8 Hz, 3H), 0.27 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 159.3, 147.9, 132.3, 130.4, 129.2, 125.8, 113.8, 98.6, 78.2, 70.3, 55.2, 52.3, 39.7, 31.7, 29.5, 29.3, 27.5, 25.7, 22.7, 18.2, 14.2, −3.9; ESI-HRMS found 545.2056 [M + Na]$^+$, calcd for $C_{27}H_{43}BrO_3SiNa$ 545.2063.

General Procedure for the Preparation of E-2-((3-(4-Hydroxybutyl)oxiran-2-yl)methylene)-4-(4-methoxyphenylmethoxy)-3-((Z)-oct-2-enyl)cyclopentanone, ***24***. To a solution of 2-bromo-3-[(1,1-dimethylethyl)dimethylsilyloxy]-4-(4-methoxyphenylmethoxy)-3-((Z)-oct-2-enyl)cyclo-pent-1-ene, e.g., **23** (1.27 g, 2.43 mmol), in diethyl ether (28 mL) was added 1.7 M *tert*-butyllithium in

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pentane (3.14 mL, 5.32 mmol) at −78 °C. The reaction mixture was stirred at −78 °C for 1 h, and then a solution of 3-(4-[(1,1-dimethylethyl)dimethylsilyloxy]butyl)oxirane-2-carboxaldehyde, e.g., **5** (0.92 g, 3.63 mmol), in diethyl ether (7 mL) was added dropwise. The reaction mixture was stirred at −78 °C for 2 h and then quenched with saturated aqueous NH_4Cl (15 mL). The aqueous layer was extracted with diethyl ether (2 × 50 mL). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4, filtered, and then concentrated to dryness in vacuo. The residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 5:1, v/v) to afford the desired diastereomeric alcohols (1.36 g, 1.95 mmol, 80%) as a pale yellow oil. To the mixture of the alcohol (1.36 g, 1.95 mmol) was added a mixture of THF/formic acid/water (20 mL, 6:3:1, v/v/v). The reaction mixture was stirred at 21 °C for 1 h and then quenched with a pH 7 buffer (30 mL). The mixture was extracted with diethyl ether (3 × 30 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ (250 mL), brine (100 mL), dried over Na_2SO_4, filtered, and then concentrated to dryness in vacuo. The residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 2:1, v/v) to afford the desired enone product, e.g., **24** (0.79 g, 1.13 mmol, 58%), as a colorless yellow oil.

(3S,4S,E)-2-(((2R,3S)/(2S,3R)-3-(4-Hydroxybutyl)oxiran-2-yl)-methylene)-4-(4-methoxyphenylmethoxy)-3-((Z)-oct-2-enyl)-cyclopentanone (Cis) ***24*** *and Its (3R,4R)-Enantiomer.* R_f = 0.48 (hexane/ethyl acetate, 1:1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.27 (d, J = 8.4 Hz, 2H), 6.88 (d, J = 8.4 Hz, 2H), 6.11 (d, J = 9.2 Hz, 1H), 5.54−5.41 (m, 2H), 4.54 (d, J = 11.4 Hz, 1H), 4.49 (d, J = 11.4 Hz, 1H), 4.16 (m, 1H), 3.78 (s, 3H), 3.63 (t, J = 6.0 Hz 2H), 3.33 (m, 1H), 3.24 (dd, J = 7.2, 2.0 Hz, 1H), 2.91 (m, 1H), 2.67−2.49 (m, 3H), 2.18 (m, 1H), 1.97 (m, 2H), 1.73−1.50 (m, 6H), 1.32−1.25 (m, 7H), 0.88 (t, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 201.7, 159.4, 143.9, 134.0, 132.9, 129.8, 129.3, 126.2, 113.9, 76.8, 62.4, 60.4, 55.3, 42.8, 41.7, 32.3, 31.5, 29.3, 27.3, 25.7, 22.5, 22.2, 14.2; ESI-HRMS found 479.2771 [M + Na]$^+$, calcd for $C_{28}H_{40}O_5Na$ 479.2773.

(3R,4R,E)/(3S,4S,E)-2-(((2R,3R)/(2S,3S)-3-(4-Hydroxybutyl)oxiran-2-yl)methylene)-4-(4-methoxyphenylmethoxy)-3-((Z)-oct-2-enyl)-cyclopentanone (Trans). R_f = 0.46 (hexane/ethyl acetate, 1:1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.20 (d, J = 8.4 Hz, 2H), 6.85 (d, J = 8.8 Hz, 2H), 6.23 (dd, J = 8.8, 1.6 Hz, 1H), 5.49 (m, 1H), 5.37 (m, 1H), 4.42 (s, 2H), 3.98 (m, 1H), 3.77 (s, 3H), 3.62 (t, J = 6.4 Hz, 2H), 3.24 (m, 2H), 2.95 (m, 1H), 2.53 (m, 2H), 2.19 (ddd, J = 14.4, 7.6, 7.6 Hz, 1H), 2.08 (ddd, J = 15.2, 7.2, 7.2 Hz, 1H), 1.94 (m, 3H), 1.72 (m, 1H), 1.58 (m, 5H), 1.29 (m, 6H), 0.88 (t, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 204.0, 159.3, 142.7, 134.1, 133.2, 129.9, 129.2, 125.4, 113.8, 70.1, 62.4, 60.3, 55.3, 55.2, 45.5, 42.5, 32.3, 31.6, 31.5, 31.4, 29.2, 29.1, 27.4, 22.5, 22.2, 14.0; ESI-HRMS found 479.2768 [M + Na]$^+$, calcd for $C_{28}H_{40}O_5Na$ 479.2773.

General procedure for the preparation of 4-[3-((E)-(3-(4-methoxyphenylmethoxy)-2-((Z)-oct-2-enyl)-5-oxocyclopentylidene]methyl)oxiran-2-yl]butanal. To a solution of *E*-2-((3-(4-hydroxybutyl)oxiran-2-yl)methylene)-4-(4-methoxyphenylmethoxy)-3-((*Z*)-oct-2-enyl)cyclopentanone (166 mg, 0.36 mmol) in dichloromethane (10 mL) at 0 °C was added solid $NaHCO_3$ (154 mg) followed by Dess−Martin periodinane (186 mg, 0.44 mmol) portionwise. The reaction mixture was allowed to warm to 21 °C and stirred for 1 h. The solution was quenched with saturated aqueous $NaHCO_3$ (10 mL) and saturated aqueous $Na_2S_2O_3$ (10 mL). The aqueous layer was extracted with dichloromethane (3 × 20 mL). The combined organic layers were dried over Na_2SO_4, filtered, and then concentrated to dryness in vacuo. The residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 3:1, v/v) to afford the desired aldehyde (140 mg, 0.31 mmol, 86%) as a colorless yellow oil.

4-[(2R,3S)/(2S,3R)-3-((E)-((2R,3R)/(2S,3S)-3-(4-Methoxyphenylmethoxy)-2-((Z)-oct-2-enyl)-5-oxocyclopentylidene]methyl)oxiran-2-yl]butanal (Cis). R_f = 0.62 (hexane/ethyl acetate, 1:1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.78 (t, J = 1.36 Hz, 1H), 7.27 (d, J = 8.8 Hz, 2H), 6.88 (d, J = 8.8 Hz, 2H), 6.12 (dd, J = 9.3, 1.6 Hz, 1H), 5.46 (m, 1H), 5.32 (m, 1H), 4.55 (d, J = 11.3 Hz, 1H), 4.49 (d, J = 11.3 Hz, 1H), 4.18 (ddd, J = 14.4, 7.4, 7.4 Hz, 1H), 3.78 (s, 3H), 3.32 (m, 1H), 3.22 (dd, J = 9.3, 2.0 Hz, 1H), 2.94 (m, 1H), 2.64−2.45 (m, 4H), 2.08 (m, 1H), 1.95 (m, 2H), 1.82 (m, 3H), 1.51 (m, 1H), 1.28 (m, 7H), 0.86 (t, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 201.6, 201.5, 159.4, 144.1, 133.6, 132.9, 129.8, 129.3, 126.2, 113.9, 75.1, 71.1, 59.9, 55.3, 55.0, 43.2, 42.8, 41.7, 31.5, 30.9, 29.3, 27.3, 25.7, 22.5, 18.4, 14.0; ESI-HRMS found 477.2611 [M + Na]$^+$, calcd for $C_{28}H_{38}O_5Na$ 477.2617.

4-[(2R,3R)/(2S,3S)-3-((E)-((2R,3R)/(2S,3S)-3-(4-Methoxyphenylmethoxy)-2-((Z)-oct-2-enyl)-5-oxocyclopentylidene]methyl)oxiran-2-yl]butanal (Trans). R_f = 0.60 (hexane/ethyl acetate, 1:1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.74 (s, 1H), 7.18 (d, J = 8.4 Hz, 2H), 6.84 (d, J = 8.8 Hz, 2H), 6.19 (d, J = 8.8 Hz, 1H), 5.48 (m, 1H), 5.36 (m, 1H), 4.41 (s, 2H), 3.96 (m, 1H), 3.75 (s, 3H), 3.22 (m, 2H), 2.92 (m, 1H), 2.51 (m, 4H), 2.19 (ddd, J = 14.0, 8.0, 8.0 Hz, 1H), 2.07 (ddd, J = 14.0, 7.0, 7.0 Hz, 1H), 1.94 (m, 2H), 1.78 (m, 3H), 1.51 (m, 1H), 1.28 (m, 6H), 0.86 (t, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 203.8, 201.6, 159.2, 148.9, 133.7, 133.2, 129.9, 129.2, 125.4, 113.8, 77.5, 70.1, 59.9, 55.2, 54.9, 45.4, 43.2, 42.5, 31.5, 31.4, 31.0, 29.2, 27.4, 22.5, 18.4, 14.0; ESI-HRMS found 477.2613 [M + Na]$^+$, calcd for $C_{28}H_{38}O_5Na$ 477.2617.

General Procedure for the Preparation of 4-[3-(E)-(3-(4-Methoxyphenylmethoxy)-2-((Z)-oct-2-enyl)-5-oxocyclopentylidene)-methyl]oxiran-2-yl]butanoic Acid, ***25****.* To a solution of 4-[3-((*E*)-(3-(4-methoxyphenylmethoxy)-2-((*Z*)-oct-2-enyl)-5-oxocyclopentylidene]methyl)oxiran-2-yl]butanal (143 mg, 0.32 mmol) in *tert*-butyl alcohol (4.8 mL) and 2-methyl-2-butene (2.88 mL) was added a solution of 80% $NaClO_2$ (215 mg, 1.89 mmol) and $NaH_2PO_4.H_2O$ (262 mg, 1.89 mmol) in water (4.8 mL) at 21 °C. The reaction mixture was stirred at 21 °C for 1 h and then quenched with water (10 mL). The aqueous layer was extracted with diethyl ether (3 × 20 mL). The combined organic layers were dried over Na_2SO_4, filtered, and then concentrated to dryness in vacuo. The residue was purified by flash column chromatography over silica gel (dichloromethane/methanol, 20:1, v/v) to afford the desired acid (123 mg, 0.26 mmol, 82%) as a colorless oil.

4-[(2R,3S)/(2S,3R)-3-((E)-((2R,3R)/(2S,3S)-3-(4-Methoxyphenylmethoxy)-2-((Z)-oct-2-enyl)-5-oxocyclopentylidene)methyl]oxiran-2-yl]butanoic Acid (Cis), ***25****, and Its Enantiomer.* R_f = 0.36 (hexane/ethyl acetate, 1:1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.28 (d, J = 8.8 Hz, 2H), 6.89 (d, J = 8.8 Hz, 2H), 6.13 (dd, J = 9.3, 1.6 Hz, 1H), 5.46 (m, 1H), 5.33 (m, 1H), 4.56 (d, J = 11.3 Hz, 1H), 4.50 (d, J = 11.3 Hz, 1H), 4.19 (ddd, J = 9.6, 7.3, 7.3 Hz, 1H), 3.79 (s, 3H), 3.32 (m, 1H), 3.23 (dd, J = 7.2, 2.0 Hz, 1H), 2.98 (m, 1H), 2.66−2.41 (m, 5H), 2.09 (m, 1H), 1.95 (m, 2H), 1.82 (m, 2H), 1.52 (m, 1H), 1.28 (m, 7H), 0.88 (t, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 201.7, 178.2, 159.4, 144.1, 133.7, 133.0, 129.8, 129.3, 126.2, 113.9, 75.1, 71.1, 59.9, 55.3, 55.0, 42.8, 41.7, 33.3, 31.5, 30.9, 29.3, 27.3, 25.7, 22.5, 21.0, 14.0; ESI-HRMS found 469.2586 [M − H]$^-$, calcd for $C_{28}H_{37}O_6$ 469.2590.

4-[(2R,3R)/(2S,3S)-3-(E)-((2R,3R)/(2S,3S)-3-(4-Methoxyphenylmethoxy)-2-((Z)-oct-2-enyl)-5-oxocyclopentylidene)methyl]oxiran-2-yl]butanoic Acid (Trans). R_f = 0.35 (hexane/ethyl acetate, 1:1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.1 (b, 1H), 7.21 (d, J = 8.4 Hz, 2H), 6.86 (d, J = 8.4 Hz, 2H), 6.23 (dd, J = 8.4, 1.6 Hz, 1H), 5.51 (m, 1H), 5.36 (m, 1H), 4.44 (s, 2H), 3.99 (m, 1H), 3.80 (s, 3H), 3.26 (m, 2H), 2.97 (m, 1H), 2.54−2.41 (m, 4H), 2.20 (ddd, J = 14.0, 7.6, 7.6 Hz, 1H), 2.09 (ddd, J = 14.0, 7.2, 7.2 Hz, 1H), 1.97 (m, 2H), 1.81 (m, 3H), 1.59 (m, 1H), 1.30 (m, 7H), 0.88 (t, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 204.0, 178.7, 159.3, 142.9, 133.8, 133.3, 129.9, 129.3, 125.4, 113.9, 77.5, 70.1, 59.9, 55.3, 55.0, 45.5, 42.5, 31.5, 31.4, 31.1, 30.3, 29.2, 27.3, 22.5, 21.1, 14.0; ESI-HRMS found 469.2582 [M − H]$^-$, calcd for $C_{28}H_{37}O_6$ 469.2590.

General Procedure for the Preparation of 4-[3-((E)-(3-Hydroxy)-2-((Z)-oct-2-enyl)-5-oxocyclopentylidene)methyl)oxiran-2-yl]butanoic Acid, El. To a solution of 4-[3-(*E*)-(3-(4-methoxyphenylmethoxy)-2-((*Z*)-oct-2-enyl)-5-oxocyclopentylidene)methyl]oxiran-2-yl]butanoic acid (137 mg, 0.29 mmol) in dichloromethane (7.3 mL) and pH 7 buffer (3.65 mL) was added DDQ (100 mg, 0.44 mmol) at 21 °C. The reaction mixture was stirred for 2 h at 21 °C and then filtered through a pad of Celite. The filtrates were washed with brine (5 mL). The aqueous layer was extracted with dichloromethane (2 × 5 mL). The combined organic layers were dried over Na_2SO_4, filtered, and then concentrated to dryness in vacuo. The residue was purified by flash

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column chromatography over silica gel (dichloromethane/methanol, 20:1, v/v) to afford the desired EI (77 mg, 0.22 mmol, 76%) as a pale yellow solid.

*4-[(2R,3S)/(2S,3R)-3-((E)-((2R,3R)/(2S,3S)-3-Hydroxy-2-((Z)-oct-2-enyl)-5-oxocyclopentylidene)methyl]oxiran-2-yl]butanoic Acid (cis-EI), **1** and **2**.* R_f = 0.36 (hexane/ethyl acetate, 1:1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.17 (d, J = 9.0 Hz, 1H), 5.50 (m, 1H), 5.38 (m, 1H), 4.54 (m, 1H), 3.25 (m, 2H), 2.98 (m, 1H), 2.64 (m, 2H), 2.44 (m, 3H), 2.16 (m, 1H), 1.95 (m, 2H), 1.80 (m, 3H), 1.58 (m, 1H), 1.33−1.25 (m, 7H), 0.86 (t, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 201.7, 178.2, 144.1, 133.2, 126.0, 118.8, 69.1, 59.9, 55.3, 44.8, 44.4, 33.3, 31.5, 31.2, 29.3, 27.4, 25.2, 22.5, 21.0, 14.1; ESI-HRMS found 349.2011 $[M - H]^-$, calcd for $C_{20}H_{29}O_5$ 349.2015.

*4-[(2R,3R)/(2S,3S)-3-(E)-((2R,3R)/(2S,3S)-3-Hydroxy-2-((Z)-oct-2-enyl)-5-oxocyclopentylidenemethyl]oxiran-2-yl]butanoic Acid (trans-EI), **3** and **4**.* R_f = 0.35 (hexane/ethyl acetate, 1:1, v/v); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.25 (d, J = 9.0 Hz, 1H), 5.50 (m, 1H), 5.36 (m, 1H), 4.36 (bd, J = 5.0 Hz, 1H), 3.29 (m, 1H), 3.11 (m, 1H), 2.98 (m, 1H), 2.64 (m, 2H), 2.44 (m, 2H), 2.26 (m, 1H), 2.11 (m, 1H), 1.95 (m, 2H), 1.81 (m, 3H), 1.59 (m, 1H), 1.32 (m, 7H), 0.86 (t, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 204.1, 178.3, 142.6, 134.4, 133.3, 125.3, 71.3, 60.0, 55.0, 48.9, 45.1, 33.3, 31.5, 31.3, 30.9, 29.2, 27.3, 22.5, 21.0, 14.0; ESI-HRMS found 349.2018 $[M - H]^-$, calcd for $C_{20}H_{29}O_5$ 349.2015.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

NMR spectra for all new compounds and an Excel file containing metadata and normalized array data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

DDQ, dichlorodicyanoquinone; DIBAL, diisobutylaluminum hydride; HAEC, human aortic endothelial cell; IL-1β, interleukin 1β; IL-8, interleukin 8; LDL, low-density lipoprotein; MCP-1, monocyte chemotactic protein 1; MEK, methyl ethyl ketone; MM-LDL, minimally modified low-density lipoprotein; Ox-PAPC, oxidized 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine; PCC, pyridinium chlorochromate; PEIPC, 1-palmitoyl-2-(5,6-epoxyisoprostane E2)-*sn*-glycero-3-phosphocholine; PMB, *p*-methoxybenzyl; EI, epoxyisoprostane; PGE2, prostaglandin E2; UPR, unfolded protein response

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