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Total Synthesis of 8,12-*iso*-iPF$_{3\alpha}$-VI, an EPA-Derived Isoprostane: Stereoselective Introduction of the Fifth Asymmetric Center

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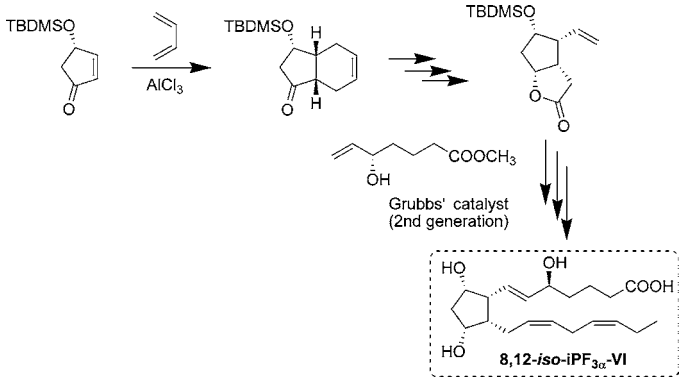

A new and stereoselective approach for the synthesis of all-syn isoprostanes is reported. This method, which is based on acid-catalyzed Diels−Alder reaction, allows the introduction of the side chain with a predetermined stereochemistry of the hydroxy group. The first total synthesis of an eicosapentaenoic acid (EPA)-derived iP, 8,12-*iso*-iPF$_{3\alpha}$-VI **10**, was performed using this approach.

Introduction

Lipid peroxidation plays an important role in the pathogenesis of several diseases such as Alzheimer's disease, atherosclerosis, cancer, and other neurodegenerative diseases.[1−4] Recently, a new class of natural products, the isoprostanes (iPs), isomeric with the enzymatically produced prostaglandins (PGs), has been discovered.[5,6] Isoprostanes are produced by a noncyclooxygenase, free radical-catalyzed peroxidation of polyunsaturated fatty acids (PUFAs). Unlike PGs (e.g., **1**) whose side chains are trans to each other, the lateral side chains of iPs (e.g., **2**−**4**) are cis in relation to the plane of prostane ring (Scheme 1).

Isoprostanes are relatively stable compounds, which makes them a good and reliable index of lipid peroxidation.[7−9] Over the past few years, we have developed strategies for the synthesis of iPs, which we have used to perform the total synthesis of arachidonic acid (AA)-derived iPs, in particular a representative of each of the four groups of iPs derived from AA (iPF$_{2\alpha}$-III **2**, iPF$_{2\alpha}$-IV, iPF$_{2\alpha}$-V, iPF$_{2\alpha}$-VI **3**) and other iP metabolites.[6,10−12]

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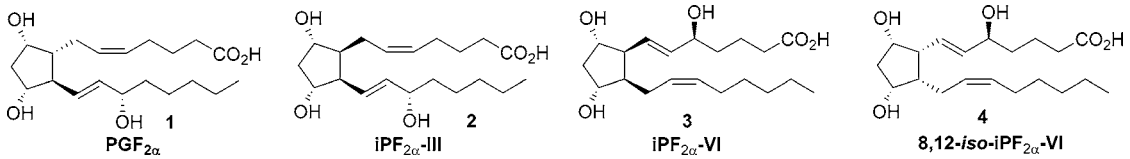
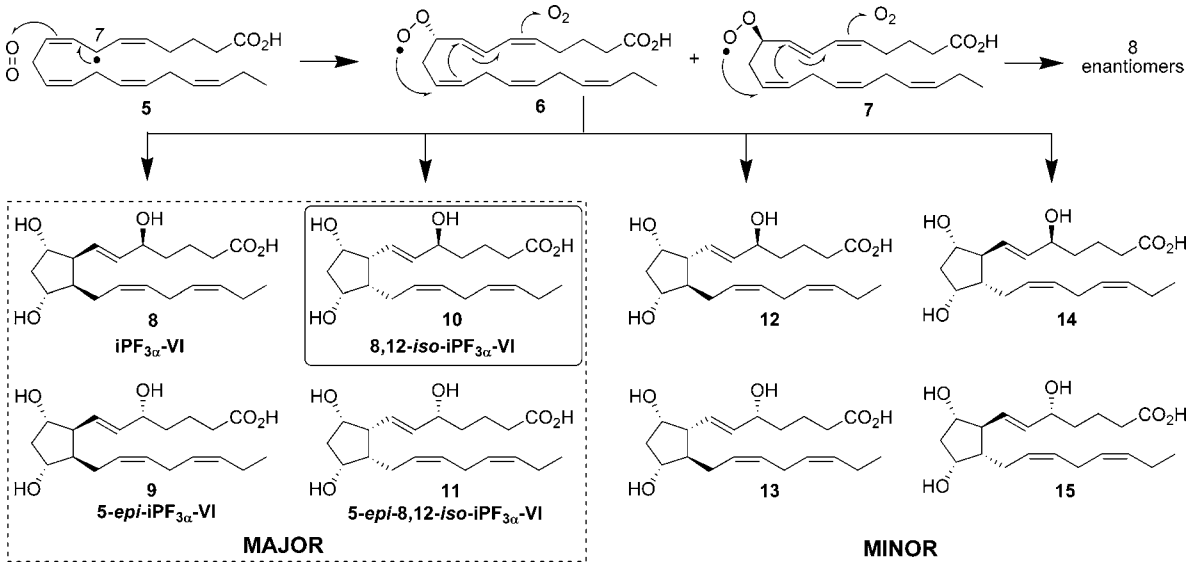
SCHEME 1



SCHEME 2. Group VI iPs Derived from EPA



Other synthetic approaches to iPs have also been reported in the literature.[13−18]

Using these synthetic standards, we have developed a GC/MS[19−21] and LC/MS/MS[22,23] methodology to analyze the distribution of individual F_2-iPs in each class in human urine and animal models. We have measured elevated urinary levels of iPs in AD patients and found that the levels correlate with the severity of the disease.[24] Equally, the level of these iPs increases with cardiovascular impairments.[25] We have also shown, in perhaps the most striking proof to date of free-radical involvement in atherosclerosis, that vitamin E suppresses iP generation in vivo and reduces atherosclerosis in apolipoprotein E (apoE) deficient mice.[26] One of the most important parameters in the study, cholesterol, remains unchanged, indicating that it is not the cholesterol that is directly responsible for the atherosclerotic lesions, but the free-radical peroxidation of PUFAs.

Of great interest are iPs derived from EPA (20:5ω-3) and docosahexaenoic acid (DHA; 22:5ω-3), which in the case of DHA have been named neuroprostanes.[27] EPA and DHA are the main PUFAs in fish and marine oils. Diets rich in fish and marine animals have been associated with a lower incidence of cardiovascular disease in Japan and Greenland.[28−30] In such population, the AA:EPA ratio has been found to be ~1.5 as compared to the average ratio of 11 in North America, and thus we hypothesize that iPs derived from EPA will also be formed in substantial amounts in such populations. Furthermore, this could also be true for people on EPA dietary supplements that are very popular and sold in pharmacies. Measurement of EPA-derived iPs, in addition to AA-derived iPs, will provide a better view of oxidant stress in these cases. Furthermore, studies on the biological implications of the EPA-derived iPs are also of importance and could be made possible with the availability of synthetic standards. Evidence for the formation of F_3-iPs by chemical peroxidation of EPA has been reported.[31] However, no synthesis of EPA-derived iPs has ever been reported. As a result, no individual iP has been identified in human biological fluids.

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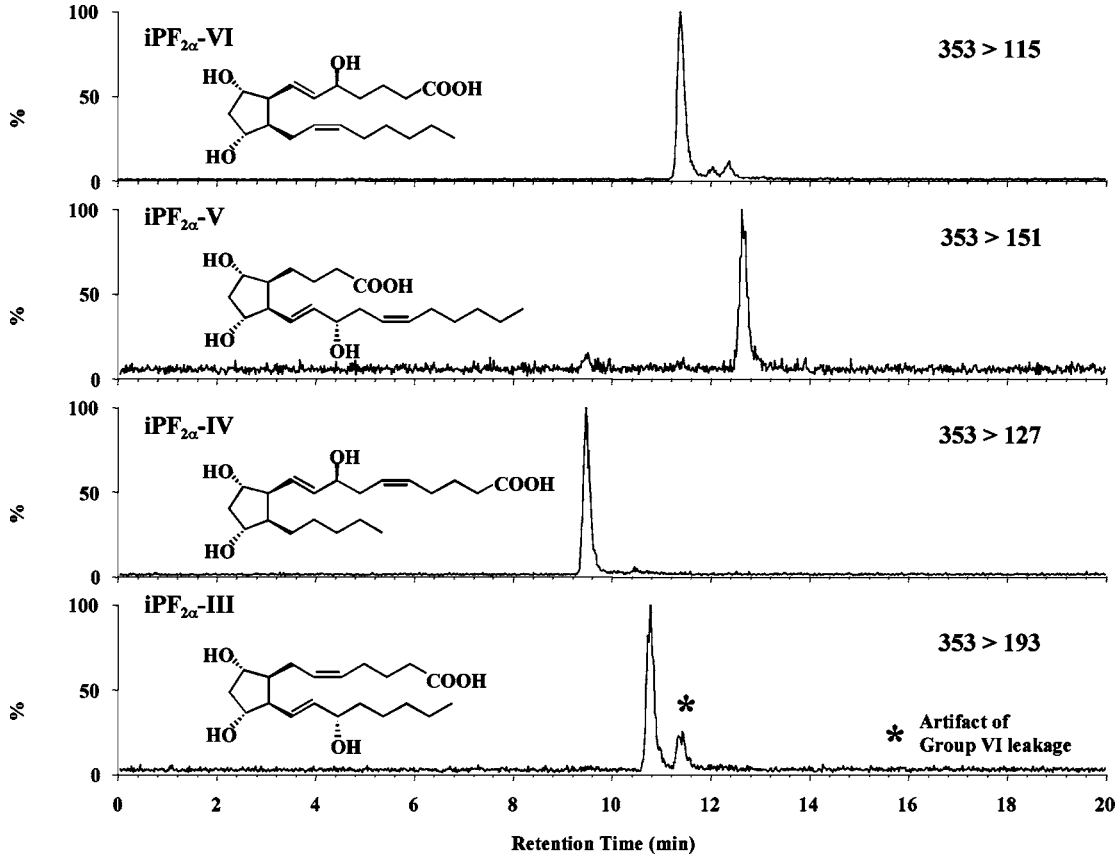

FIGURE 1. HPLC/MS/MS of a mixture of four synthetic iPs. Representatives of each of the four F_2-iP classes were mixed and analyzed during a single multiple reaction-monitoring LC/MS/MS run.

We herein report a novel and stereoselective approach to all-syn iPs (the two OH groups and the two side chains are on the same face of the prostane ring), for example, **4**. To demonstrate our approach, which is based on acid-catalyzed Diels−Alder reaction, the first total synthesis of 8,12-*iso*-iP$F_{3\alpha}$-VI **10**, an all-syn EPA-derived iP of group VI, was performed. As shown in Scheme 2, there are a total of 16 group VI iPs that can be generated from EPA by an endoperoxide mechanism.[32]

Results and Discussion

The selection of **10** as a synthetic target is based on our previous discovery that the two all-syn AA-derived iPs, 8,12-*iso*-iP$F_{2\alpha}$-VI **4** and its epimer, 5-*epi*-8,12-*iso*-iP$F_{2\alpha}$-VI, are the most abundant iPs discovered in human urine (Figure 2).[20] Also, a previous report on the radical peroxidation of linolenic acid shows a preponderance of all-syn products as compared to the syn−anti−syn isomers (the two hydroxy groups are cis to each other and anti to the two side chains).[33] Because the structural differences between AA and EPA are minimal, it is not unreasonable to expect that all-syn iP **10** and **11** will also be the major iPs derived from EPA.

Synthetic Approach. We have previously reported a thermal Diels−Alder procedure for the preparation of syn−anti−syn iPs, for example, **2** and **3**.[11] In the present case, we are interested in developing a flexible route to all-syn iPs. Because acid-catalyzed Diels−Alder reaction is known to favor products from a syn approach,[11,34] we decided to design a synthetic route based on it. As shown in Scheme 3, using 1,3-butadiene **21** as the diene component, an all-syn tetrasubstituted intermediate such as **18** can be generated wherein the two functional groups in the prostane ring are the same but can be differentiated to allow the introduction of different top and bottom side chains.

Another important objective of this synthesis is an attempt to introduce the side chain with a predetermined stereochemistry of the hydroxy group. In our previous syntheses of iPs, we have constructed the stereochemistry of the hydroxy group in the side chain via the BINAL-H reduction of its keto-derivative.[10,11] We have found this process to be very useful and result in good ee's. However, the process is not universal and has exceptions,[35,36] and, in such cases, the identity of the product is in doubt. Thus, development of an approach to introduce the side chain with predetermined stereochemistry of the hydroxy group is of importance. In the present case, we have envisioned that this could be accomplished by a crossed olefin metathesis between prostane ring synthons such as **16** or **17** and the side chain intermediate. By design, synthons **16** and **17** already contain substitutions with the required stereochemistry, in this case, all-syn, necessary for the construction of the top and bottom side chains. The use of such intermediates for the

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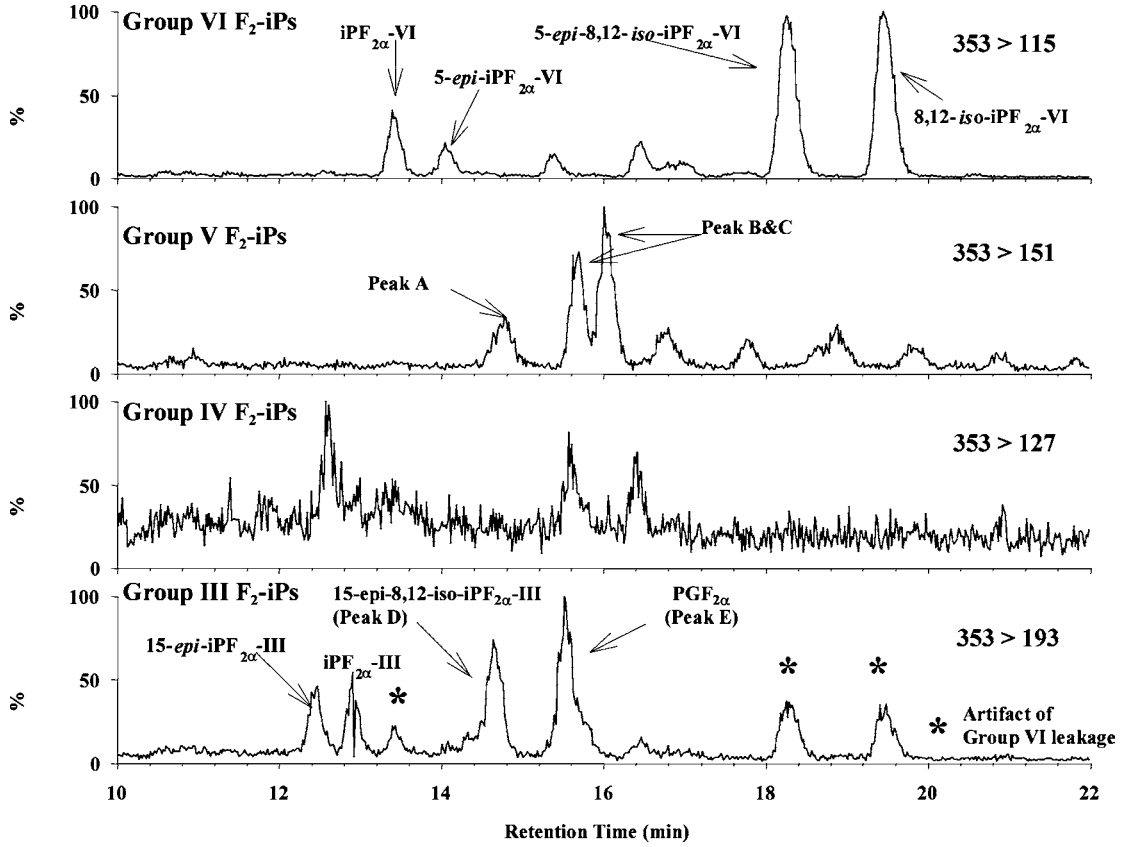
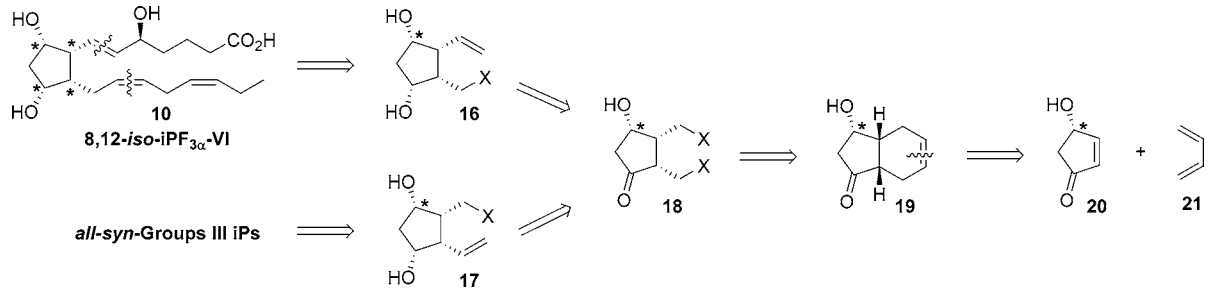


FIGURE 2. HPLC/MS/MS of urinary iPs. Selected peaks were identified by comparison with synthetic standards.

SCHEME 3. Retrosynthetic Analysis



introduction of side chains containing preformed hydroxy groups has never been attempted before.[37] An advantage of this approach is that it is not specifically designed for a certain iP group and thus offers a large degree of flexibility in the synthesis of iPs. As can be seen, the chirality of the hydroxy group in **20** determines the stereochemistry of the four substituents on the five-membered ring.

Preparation of ene-Lactone 33. 4(*S*)-Hydroxycyclopent-2-enone **20** was prepared as described by us previously.[38,39] Protection of **20** with TBDMSCl was done in preference to TBDPSCl.[11,34] Diels−Alder reaction of dienophile **22** and 1,3-butadiene **21** in toluene catalyzed by $AlCl_3$ afforded the desired all-syn product **23** and the syn−anti−syn product (not shown) in 70% combined yield.[11,34] About 8% of the deprotected derivatives of **23** and the syn−anti−syn product was also isolated. With the recovered 8%, the total yield is ∼78%.

Oxidation of **23** with a catalytic amount of OsO_4, in the presence of trimethylamine-*N*-oxide, afforded **24** in 90% yield (Scheme 4). A small quantity of its isomer (∼9%) **25**, a product generated from the approach from the more hindered concave face, was also isolated. Cleavage of the diol **24** by $NaIO_4$ in the presence of ruthenium chloride hydrate afforded bis-acid **26** in 95% yield. Probably because of steric hindrance, a much slower oxidation was observed for **25**, wherein intermediate aldehyde-acids were isolated. Bis-acid **26** was esterified with diazomethane to give **27** in 91% yield.

The success of this approach depends on whether we can differentiate the two generated ester groups in **27** in Scheme 4. This was accomplished through the stereocontrolled reduction of the keto-group. We have chosen the bulky L-Selectride as reducing agent to ensure a hydride attack from the β face, which we anticipated to be the less crowded face, to yield the desired α-hydroxy intermediate. The L-Selectride reduction of the keto-

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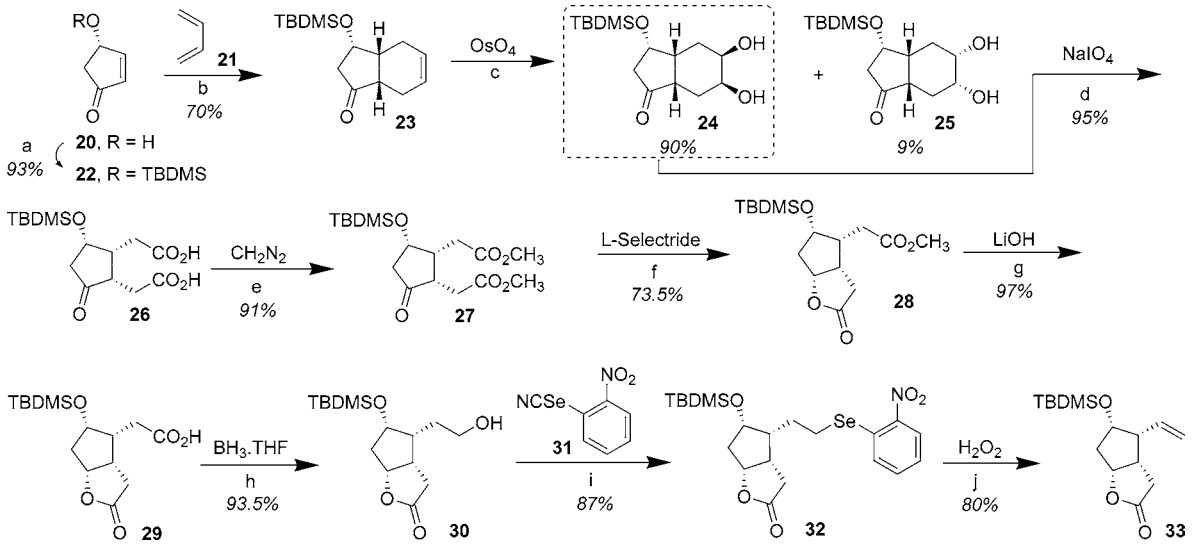
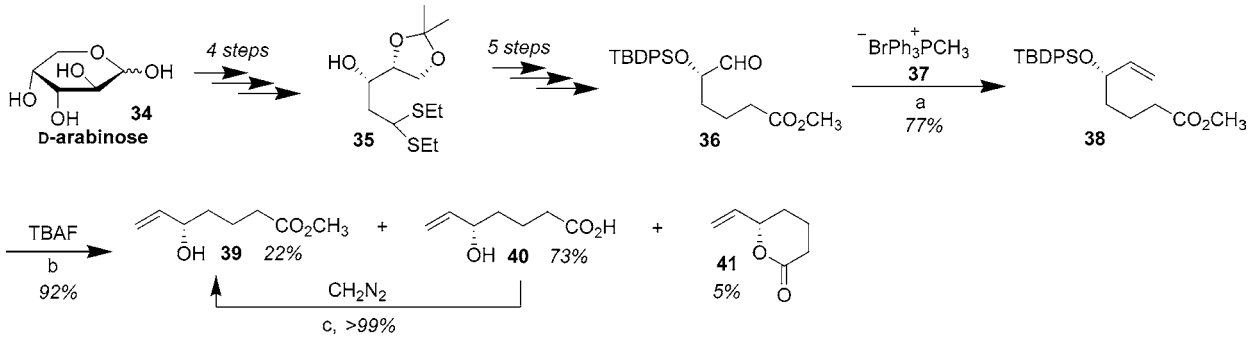
SCHEME 4. Preparation of ene-Lactone 33[a]



[a] Reagents and conditions: (a) TBDMSCl, Et_3N, DMAP, CH_2Cl_2, rt, 8 h; (b) $AlCl_3$, toluene, rt, 1.5 h; (c) $(CH_3)_3N(O)\cdot 2H_2O$, *t*-BuOH/CH_3CN/H_2O (21:11:5), rt, 1 h; (d) $RuCl_3\cdot xH_2O$, CH_3CN/CCl_4/H_2O (2:2:3), rt, 2.5 h; (e) Et_2O, rt, 1 h; (f) THF, −78 °C (30 min) to −40 °C (45 min); (g) *i*-PrOH, rt, 2.5 h; (h) THF, 0 °C (30 min) to rt (1 h); (i) *n*-Bu_3P, THF, rt, 8 h; (j) THF, rt, 3 h.

SCHEME 5. Preparation of Top Side Chain Synthon 39[a]



[a] Reagents and conditions: (a) NaHMDS, THF, −60 °C to rt, 1.25 h; (b) THF, rt, 12 h; (c) Et_2O, rt, 1 h.

group afforded **28** in 73.5% yield. The five-membered ring lactone in **28** is formed in the same step. The formation of a bottom lactone also confirms that we have an intermediate whose substitutions are all on the same face (all-syn) in reference to the plane of the ring.

Hydrolysis of the methyl ester using LiOH in 2-propanol afforded **29** in 97% yield. It is possible that the LiOH also hydrolyzed the lactone. Under workup conditions, the lactone is automatically re-formed. To selectively reduce the carboxy acid in the presence of the lactone, we used BH_3•THF as reducing agent. The reaction went well to afford **30** in 93.5% yield. The alcohol was converted to ene-lactone **33** in two steps. The first step involved the derivatization of the alcohol using *o*-nitrophenylselenocyanate **31** to form intermediate **32** in 87% yield.[40] Oxidation of **32** with H_2O_2 in THF afforded **33** in 80% yield. In addition to the chemical transformations, the confirmation of the structural assignment of **33** is provided by ^1H NMR, ^{13}C NMR, IR, MS data, decoupling studies, and NOESY (see Supporting Information).

Preparation of the Top Side Chain Synthon 39. We have designed the synthesis of top side chain synthon **39**, shown in Scheme 5, in such a way that it is amenable to large scale preparation and, at the same time, the hydroxy group will have an optically pure "*S*" stereochemistry. These prerequisites were satisfied by using cheap and commercially available D-arabinose **34**. Synthesis of **36** from D-arabinose was done in nine steps, as described by us previously.[41] **36** was coupled with the phosphorane derived from the commercially available one-carbon phosphonium salt **37** to give **38** in 77% yield. Desilylation of **38** with TBAF afforded a mixture of hydroxy-ester **39** (∼22%), hydroxy-acid **40** (73%), and lactone **41** (5%), with a combined yield of 92%. **39**, **40**, and **41** can be easily purified and separated by column chromatography. **40** was esterified back to **39** using diazomethane in quantitative yield.

Synthesis of 8,12-*iso*-iPF$_{3\alpha}$-VI. The completion of the synthesis of 8,12-*iso*-iPF$_{3\alpha}$-VI **10** is shown in Scheme 6. As mentioned earlier, we have planned to introduce the top side chain of **10** by crossed olefin metathesis. The metathesis reaction between ene-lactone **33** and the top side chain synthon **39** was performed in CH_2Cl_2 at room temperature and was catalyzed by Grubbs' catalyst (2nd generation). The reaction was allowed to run for 3−4 days, and addition of 1 equiv of **39** and 20−

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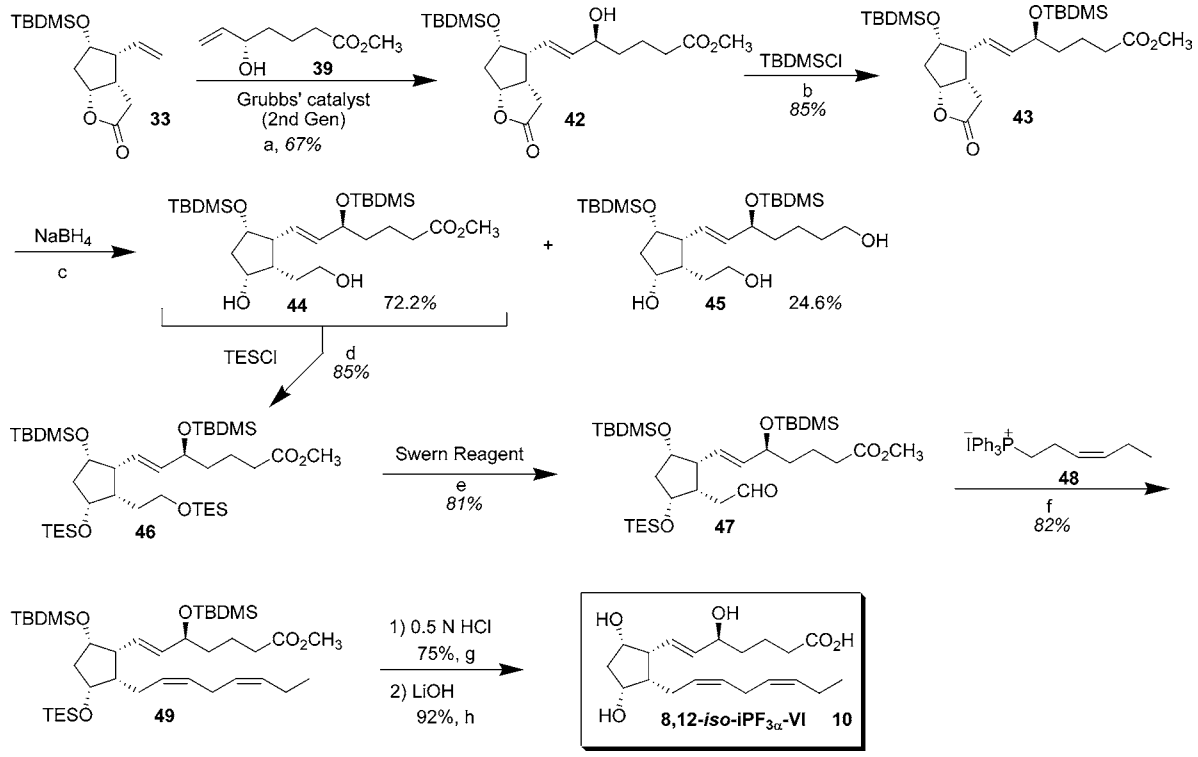
SCHEME 6. Synthesis of 8,12-*iso*-iPF$_{3\alpha}$-VI 10[a]



[a] Reagents and conditions: (a) CH_2Cl_2, rt, 3−4 days; (b) Et_3N, DMAP, CH_2Cl_2, reflux, 3.5 h; (c) ether/MeOH, rt, 35 min; (d) Et_3N, DMAP, CH_2Cl_2, reflux, 2.5 h; (e) CH_2Cl_2, −70 °C to −50 °C, total time = 2 h; (f) NaHMDS, THF, −60 °C (30 min) to rt (30 min); (g) THF, rt, 8 h; (h) ^{i}PrOH/H_2O, rt, 3 h.

30% mol of Grubbs' catalyst was done each day to afford **42** in 67% yield. As expected, the dimerization product of **39** was also formed. Protection of **42** with TBDMSCl afforded **43** in 85% yield.

Reduction of **43** with DIBAL-H at −78 °C afforded the lactol derivative (not shown) in 60% yield. Wittig reaction of the lactol and the phosphorane derived from phosphonium salt **48**[42] failed to produce the desired product, and lactol was recovered unchanged. With all of the substituents of the prostane ring on the same face and the presence of silyl protecting groups, Wittig reaction of such sterically hindered lactol may not be possible. Thus, we decided to reduce the lactone completely using $NaBH_4$ to form diol **44**. Bis-silylation with TESCl afforded **46** in 85% yield. One step conversion of **46** to aldehyde **47** was accomplished in 81% yield using Swern oxidation, which greatly favored the oxidation of the primary silyl group.[43] Coupling of **47** with the phosphorane generated from **48** afforded **49** in 82% yield. The removal of the tris-silyl groups in **49** was performed in THF using 0.5 N HCl. Finally, hydrolysis with LiOH in 2-propanol afforded the desired compound, 8,12-*iso*-iPF$_{3\alpha}$-VI **10**, in 92% yield.

As can be seen from Schemes 1 and 2, there is only a slight structural difference between F_2-iPs and F_3-iPs. We are hypothesizing that the mass spectral fragmentation of F_3-iPs will be very similar to those of F_2-iPs. In the past, we have shown that each group of F_2-iP has an abundant fragment ion, which is specific to that group and not present in the others. For example, *m*/*z* 115 is the characteristic fragment ion of group VI, *m*/*z* 151 for group V, *m*/*z* 127 for group IV, and *m*/*z* 193 for group III (Figure 1).[23] Using this information, we were able to establish an assay that allows simultaneous analysis of selected isomers of each of the four groups using high performance liquid chromatography/electrospray/tandem mass spectrometry (HPLC/ESI/MS/MS) through selective ion monitoring. The high specificity of each fragment ion is illustrated in the good mass spectrometric resolution shown in Figure 1. Chromatograms of groups IV, V, and VI are not contaminated with any peaks from other groups. The chromatogram of group III contains a slight contamination from group VI. This does not create any ambiguities in the analysis because the peaks of groups III and VI are well resolved. With selective ion monitoring, we were able to measure with one injection the urinary profile of the four groups of AA-derived iPs as shown in Figure 2.

Figure 3 shows the mass spectrum of 8,12-*iso*-iPF$_{3\alpha}$-VI **10**. As anticipated, an ion at *m*/*z* 115 dominated the fragment ion spectrum as was the case for iPF$_{2\alpha}$-VI **3**.[23] The selective ion monitoring at *m*/*z* 351 (molecular ion peak) and at ion fragment *m*/*z* 115 in the case of group VI guarantees the noninterference by *m*/*z* 115 of F_2-iPs. These preliminary results are promising, and we are hopeful that, as in the case of F_2-iPs, we will be able to develop a sensitive LC/MS/MS methodology to analyze and measure the distribution EPA-derived iPs in biological fluids.

Conclusion

In summary, we have described an interesting variation of our Diels−Alder approach to isoprostanes. A key feature of this approach is the introduction of the side chain containing a

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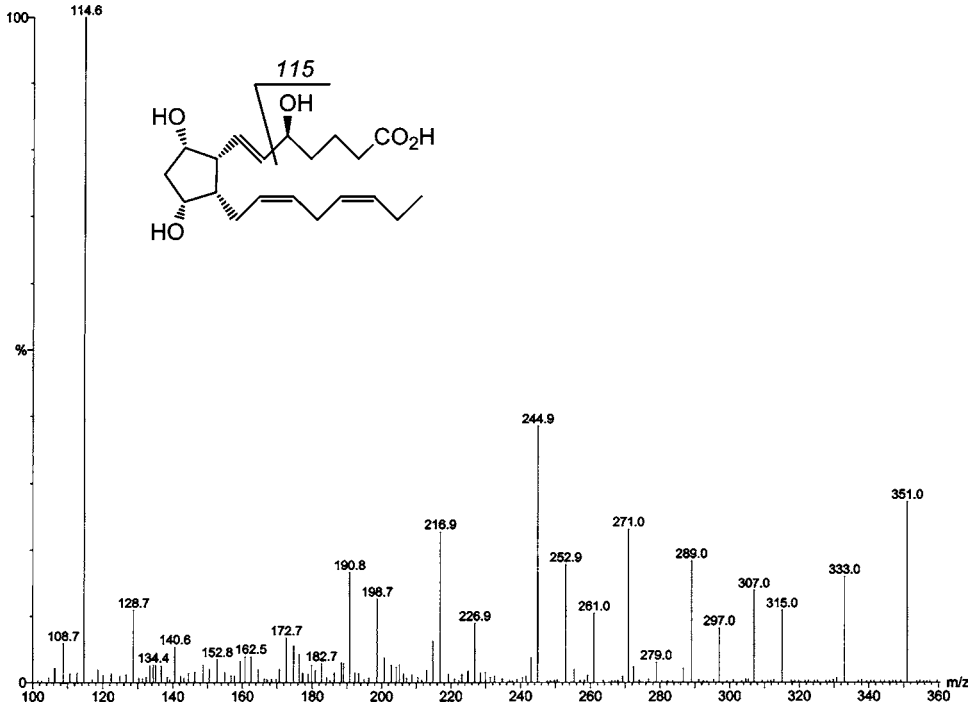

FIGURE 3. Fragment ion spectra of 8,12-*iso*-iP$F_{3\alpha}$-VI.

hydroxy group with predetermined stereochemistry. The first total synthesis of an EPA-derived iP, 8,12-*iso*-iP$F_{3\alpha}$-VI **10**, was performed using this approach. The identification of a major fragment ion *m*/*z* 115 bodes well for the development of a quantitative method for the measurement of EPA-derived iPs. This is the first step in the evaluation of EPA-derived iPs and lipid peroxidation in humans. Work is in progress for the identification of this iP in human urine.

Experimental Section

Dihydroxylation of (1*S*,6*R*,7*S*)-7-[(*tert*-Butyldimethylsilyl)oxy]-bicyclo[4.3.0]non-3-en-9-one (24). To **23** (0.085 g, 0.319 mmol) in the *t*-BuOH/CH_3CN/H_2O solvent system (2.5 mL; 21:11:5) was added $(CH_3)_3N(O)\cdot 2H_2O$ (0.107 g, 0.959 mmol). The resulting solution was treated with OsO_4 (2.5% in *t*-BuOH, 0.0892 mL). After the mixture was stirred for 1 h at room temperature, the solvent was evaporated and the remaining aqueous layer was extracted with ethyl ether (3 × 7 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. Purification of the residue by silica gel chromatography with 5% MeOH in CH_2Cl_2 as eluent afforded **24** (86.5 mg, 90%) and **25** (9.1 mg, 9%), both white solids.

Spectral Data of (1*S*,3*S*,4*R*,6*R*,7*S*)-3,4-Dihydroxy-7-[(*tert*-butyldimethylsilyl)oxy]bicyclo[4.3.0]non-3-en-9-one (24). ^{1}H NMR ($CDCl_3$) δ 4.52 (1H, qt, J = 7.4, 6.4), 4.024 (1H, br), 3.578 (1H, m), 2.62−2.78 (1H, m), 2.54 (1H, dd, J = 11.49, 7.75), 1.91−2.25 (3H, m), 1.74−1.91 (1H, m), 1.21−1.37 (1H, m), 0.891 (9H, s), 0.098 (3H, s), 0.069 (3H, s); ^{13}C NMR ($CDCl_3$) δ 216.6, 70.0, 68.9, 48.6, 44.5, 36.6, 27.3, 26.0, 25.4, 18.2, −4.731. IR (cm^{-1}) 3329.16, 1741.68; HRMS calcd for $C_{15}H_{29}O_4Si$ $[M + H]^+$ = 301.1835, obsd 301.1827.

Spectral Data of (1*S*,3*R*,4*S*,6*R*,7*S*)-3,4-Dihydroxy-7-[(*tert*-butyldimethylsilyl)oxy]bicyclo[4.3.0]non-3-en-9-one (25). ^{1}H NMR δ 4.50 (1H, t), 4.02−4.16 (2H, m), 2.64 (1H, dd, J = 6.08, 13.58), 2.33−2.49 (2H, m), 1.84−2.10 (5H, m), 0.894 (9H, s), 0.083 (2 × 3H, 2s); ^{13}C NMR ($CDCl_3$) δ 215.8, 69.9, 69.3, 68.2, 45.6, 35.2, 26.0, 26.0, 18.1, −4.6.

[3-(*tert*-Butyl-dimethylsilyl)oxy-2-carboxymethyl-5-oxo-cyclopentyl]acetic Acid (26). To a stirred solution of **24** (0.420 g, 1.4 mmol) in the $CH_3CN/CCl_4/H_2O$ solvent system (total volume = 21 mL; 2:2:3) were added $NaIO_4$ (2.38 g, 11.12 mmol) and $RuCl_3\cdot xH_2O$ (6.39 mg, 0.0308 mmol). After being stirred at room temperature for 2.5 h, the reaction mixture was diluted with CH_2Cl_2 (10 mL). Layers were separated. The aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated under vacuum. Purification by silica gel chromatography with 10−15% MeOH/CH_2Cl_2 as eluent afforded **26** (0.4363 g, 95%). ^{1}H NMR ($CDCl_3$) δ 4.40−4.51 (1H, br), 2.15−3.10 (8H, m), 0.88 (9H, s), 0.07 (3H, s), 0.12 (3H, s); IR (cm^{-1}) 1751.92, 1721.19; MS (EI) for $C_{15}H_{26}O_6Si$ $[M]^+$, 330.16.

[3-(*tert*-Butyldimethylsilyl)oxy-2-methoxycabonylmethyl-5-oxo-cyclopentyl]acetic Acid Methyl Ester (27). CH_2N_2 in ether was added to **26** (0.214 g, 0.60 mmol) at 0 °C. The light yellow solution was stirred at room temperature for 45 min. The ether was evaporated, and the residue was purified by silica gel chromatography with 5% ethyl acetate in hexane as eluent to afford **27** (211.9 mg, 91%). ^{1}H NMR ($CDCl_3$) δ 4.50−4.58 (1H, br), 3.71 (3H, s), 3.69 (3H, s), 2.88−3.0 (1H, m, br), 2.18−2.73 (7H, m), 0.88 (9H, s), 0.055 (3H, s), 0.045 (3H, s); ^{13}C NMR ($CDCl_3$) δ 216.3, 173.0, 173.0, 70.9, 52.2, 51.9, 47.1, 46.6, 41.2, 32.3, 29.9, 25.9, 18.1, −4.6, −5.1; IR (cm^{-1}) 1735.92 (br); MS (EI) for $C_{17}H_{32}O_6Si$ $[M]^+$, 358.86.

The diazomethane was prepared from commercially available 1-methyl-3-nitro-1-nitrosoguanidine (MNNG) as follows: At 0 °C, 400 mg of MNNG was slowly added to the mixture of 4 mL of 40% KOH and 10.0 mL of ethyl ether. The reaction was allowed to stir for ∼5 min. The yellow ether layer was transferred to a flask containing 1−2 pellets of KOH and allowed to stand for a minute. The resulting yellow solution was subsequently used for esterification.

(3a*S*,4*R*,5*S*,6a*R*)-Methyl-[5-(*tert*-butyldimethylsilyl)oxy-2-oxo-hexahydro-cyclopenta[*b*]furan-4-yl]acetate (28). To a solution of **27** (0.155 g, 0.4329 mmol) in THF (3.5 mL) was added L-Selectride (0.5628 mL, 1 M in THF) at −78 °C. The reaction mixture was stirred at −78 °C for 30 min and then warmed gradually to −40

°C. After being stirred for 45 min at −40 °C, the reaction was quenched with water (5 mL). The aqueous layer was extracted with ethyl acetate (3 × 7 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. Purification of the residue with 10% ethyl acetate as eluent afforded **28** (0.142 g, 73.5%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 5.098 (1H, t, J = 7.15), 4.251 (1H, m), 3.701 (3H, s), 3.065−3.191 (1H, m), 2.579−2.707 (2H, m), 2.410−2.559 (2H, m), 2.270−2.384 (1H, m), 2.223 (1H, d, J = 15.19), 1.842−1.980 (1H, m), 0.879 (9H, s), 0.068 (3H, s), 0.027 (3H, s); ^{13}C NMR ($CDCl_3$) 177.4, 173.8, 83.0, 75.0, 51.8, 42.8, 42.0, 40.2, 31.3, 30.8, 25.9, 18.2, −5.8, −6.0; IR (cm^{-1}) 1770.87, 1735.92; HRMS calcd for $C_{16}H_{29}O_5Si$ [M + H]$^+$, 329.1784; obsd, 329.1790.

(3a*S*,4*R*,5*S*,6a*R*)-[5-(*tert*-Butyldimethylsilyl)oxy-2-oxo-hexahydro-cyclopenta[*b*]furan-4-yl]acetic Acid (29). To a stirred solution of ester **28** (0.110 g, 0.335 mmol) in *i*-PrOH (2.3 mL)/H_2O (2.2 mL) was added LiOH (40.2 mg, 1.68 mmol). The solution was stirred at room temperature for 2.5 h. The reaction was quenched with 0.250 mL of 20% HCl. The aqueous layer was extracted with EtOAc (3 × 1.0 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated under vacuum. Purification of the residue with 2% MeOH/CH_2Cl_2 afforded **29** (0.101 g, 97%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 5.108 (1H, t, J = 7.3), 4.272 (1H, t), 3.093−3.266 (1H, m), 2.446−2.776 (4H, m), 2.269−2.400 (1H, m), 2.186 (1H, d, J = 15.4), 1.873−2.023 (1H, m), 0.883 (9H, s), 0.079 (3H, s), 0.046 (3H, s); ^{13}C NMR δ 178.0, 177.7, 84.7, 84.8, 75.1, 43.6, 42.1, 40.1, 31.2, 30.9, 25.9, 18.1, −4.5, −5.2; IR (cm^{-1}) 1762.16, 1700.71; HRMS calcd for $C_{15}H_{26}O_5SiNa$ [M + Na]$^+$, 337.1147; obsd, 337.1433.

(3a*S*,4*R*,5*S*,6a*R*)-Hexahydro-5-(*tert*-butyldimethylsilyl)oxy-4-(2-hydroxy-ethyl)-cyclopenta[*b*]furan-2-one (30). To a solution of **29** (0.0238 g, 0.076 mmol) in THF (1.0 mL) at 0 °C was added BH_3·THF (1.0 M, 0.121 mL). The colorless solution was stirred at 0 °C for 30 min and allowed to warm to room temperature and was stirred at this temperature for another hour. The reaction was quenched with 0.5 mL of H_2O. The aqueous layer was extracted with EtOAc (5 × 1.0 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated under vacuum. Purification of the residue by silica gel chromatography with 2% MeOH/CH_2Cl_2 as eluent afforded **30** (0.0212 g, 93.5%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 5.089 (1H, t, J = 7.4), 4.819 (1H, t, J = 3.3), 3.622−3.809 (2H, m), 2.987−3.135 (1H, m), 2.7545 (1H, dd, J = 13.2, 5.2), 2.4865 (1H, dd, J = 11.8, 6.6), 2.105−2.231 (1H, d, J = 15.2), 2.020−2.100 (1H, m), 1.821−1.950 (2H, m), 1.667−1.790 (1H, m), 0.880 (9H, s), 0.079 (3H, s), 0.060 (3H, s); ^{13}C NMR ($CDCl_3$) δ 178.0, 84.9, 75.4, 61.5, 44.5, 42.3, 40.1, 30.8, 29.4, 25.9, 18.2, −4.4, −5.1; IR (cm^{-1}) 3457.18, 1764.80; HRMS calcd for $C_{15}H_{28}O_4SiNa$ [M + Na]$^+$, 323.1649; obsd, 323.1641.

(3a*S*,4*R*,5*S*,6a*R*)-Hexahydro-5-(*tert*-butyldimethylsilyl)oxy-4-(2-(2-nitrophenylselanyl)-ethyl)-cyclopenta[*b*]furan-2-one (32). To a solution of **30** (0.090 g, 0.302 mmol) in THF (3.0 mL) were added *o*-nitrophenylselenocyanate **31** (0.343 g, 1.5 mmol) and *n*-Bu_3P (0.376 mL, 1.50 mmol) at room temperature. The reaction mixture was stirred at room temperature for 8 h. The solvent was evaporated under reduced pressure, and the residue was purified by silica gel chromatography with 20% ethyl acetate in hexane as eluent to afford **32** (0.1262 g, 87%) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ 8.310 (1H, d, J = 8.2), 7.470−7.590 (2H, m), 7.351 (1H, t, J = 7.4), 5.110 (1H, t, J = 7.2), 4.231 (1H, br), 3.021−3.165 (1H, m), 2.851−3.018 (2H, m), 2.721 (1H, dd, J = 18.2, 4.6), 2.430−2.580 (1H, dd, J = 18.2, 11.8), 1.820−2.210 (5H, m), 0.88 (9H, s), 0.079 (3H, s), 0.046 (3H, s); ^{13}C NMR ($CDCl_3$) δ 177.5, 147.2, 133.9, 129.2, 126.8, 125.9, 84.7, 74.9, 47.9, 42.2, 30.6, 25.9, 25.8, 24.6, 18.2, −4.5, −5.2; IR 1762.16; HRMS calcd for $C_{21}H_{31}O_5NSeSiNa$ [M + Na]$^+$, 508.1029; obsd, 508.1071.

(3a*S*,4*R*,5*S*,6a*R*)-Hexahydro-5-(*tert*-butyldimethylsilyl)oxy-4-vinyl-cyclopenta[*b*]furan-2-one (33). To a solution of **32** (0.096 g, 0.199 mmol) in THF (0.720 mL) was added H_2O_2 (0.220 mL, 30% (w/w) solution, commercial). The resulting solution was stirred at room temperature for 3 h. The reaction mixture was diluted with ethyl ether (1.5 mL) and 0.5 mL of H_2O. Layers were separated. The aqueous layer was extracted with ether (3 × 1.0 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated under vacuum. The residue was purified by silica gel chromatography with 10% ethyl acetate in hexane as eluent to afford **33** (0.0446 g, 80%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 6.02 (1H, ddd, J = 17.5, 10.5, 8.3), 5.23 (1H, ddd, J = 10.4, 1.9, 0.7), 5.18 (1H, ddd, J = 17.3, 1.9, 1.1), 5.11 (1H, t, J = 7.3), 4.230 (1H, t. J = 3.6), 3.10 (1H, dtd, J = 12.2, 8.3, 4.9), 2.87 (1H, dd, J = 18.6, 4.9), 2.54 (1H, td, J = 8.4, 3.5), 2.52 (1H, dd, J = 18.6, 11.9), 2.19 (1H, d, J = 15.1), 1.93 (1H, dddd, J = 15.0, 6.8, 3.7, 0.4), 0.877 (9H, s), 0.062 (3H, s), 0.044 (3H, s); ^{13}C NMR ($CDCl_3$) δ 177.8, 135.1, 118.3, 85.0, 77.0, 52.2, 42.5, 41.8, 31.3, 25.9, 18.2, −4.5, −5.0; HRMS calcd for $C_{15}H_{26}O_3Si$ [M + H]$^+$, 283.1729; obsd 283.1724.

Methyl-5(*S*)-(*tert*-butyldiphenylsilyl)oxy-hept-6-enoate (38). To a stirred solution of **37** in dry THF (15.5 mL) was added sodium hexamethyldisilazide (3.0 mL, 1.0 M in THF) at −60 °C. The reaction was stirred at this temperature for 15 min, and aldehyde **36** (0.446 g, 2.7 mmol) in dry THF (10.0 mL) was added at −60 °C. The reaction mixture was warmed to room temperature and stirred at this temperature for 1.25 h. The reaction was quenched with a saturated solution of NH_4Cl (10 mL) and extracted with ethyl acetate (3 × 15 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated under vacuum. The residue was purified by silica gel chromatography with 5% ethyl acetate in hexane as eluent to afford **38** (0.342 mg, 77%). ^{1}H NMR ($CDCl_3$) δ 7.608−7.730 (4H, m), 7.308−7.469 (6H, m), 5.716−5.848 (1H, s), 4.939−5.051 (2H, m), 4.161 (1H, qt, J = 5.3), 3.634 (3H, s), 2.172 (2H, t, J = 7.17), 1.392−1.671 (4H, m), 1.069 (9H, s); ^{13}C NMR ($CDCl_3$) δ 174.3, 140.9, 136.2 (2 C), 134.5 (2 C), 129.7 (2 C), 127.5 (2 C), 114.8, 74.5, 51.7, 37.0, 34.2, 27.2, 20.0, 19.5.

Methyl-5(*S*)-hydroxy-hept-6-enoate (39). To a stirred solution of **38** (726.4 mg, 1.83 mmol) in THF (5.5 mL) was added TBAF (2.75 mL, 1.0 mL in THF). The reaction mixture was stirred at room temperature overnight. The reaction was quenched with a saturated solution of NH_4Cl solution (10 mL) and extracted with ethyl acetate (3 × 15 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated under vacuum to give **39** (60.73 mg), **40** (176.5 mg), and **41** (11.62 mg) in 92% combined yield. **40** was esterified back to **39** using diazomethane prepared as described above. ^{1}H NMR ($CDCl_3$) δ 5.78−5.93 (1H, s), 5.19 (2H, dd, J = 17.1, 10.4), 4.161 (1H, qt, J = 6.1), 3.671 (3H, s), 2.35 (2H, t, J = 7.2), 1.453−1.821 (4H, m); ^{13}C NMR ($CDCl_3$) δ 171.2, 136.3, 117.1, 80.4, 51.1, 29.8, 28.2, 18.3.

(3a*S*,4*R*,5*S*,6a*R*)-7-[5-(*tert*-Butyl-dimethylsilyl)oxy-2-oxo-hexahydo-cyclopenta[*b*]furan-4-yl]-5-hydroxy-hept-6-enoic Acid Methyl Ester (42). To a stirred solution of **33** (0.025 g, 0.0882 mmol) and Grubbs' catalyst (2nd generation, 0.019 g, 0.0222 mmol) in CH_2Cl_2 (1.0 mL) at room temperature was added **39** (0.028 g, 0.1774 mmol). The reaction was stirred at room temperature for 4 days. One equivalent of **39** and 20−30% of the catalyst were added each day. On the fourth day, the solvent was evaporated and the residue was purified by silica gel chromatography using 2% MeOH in CH_2Cl_2 as eluent to afford **42** (20 mg, 67%). The yield was based on the recovered starting material (5.4 mg). ^{1}H NMR ($CDCl_3$) δ 6.765−5.881 (1H, dd, J = 15.5, 8.6), 5.513−5.604 (1H, dd, J = 15.5, 5.7), 5.090 (1H, t), 4.104−4.211 (2H, m), 3.676 (3H, s), 2.971−3.122 (1H, m), 2.829 (1H, dd, J = 18.3, 4.9), 2.388−2.569 (2H, m), 2.321 (2H, t, J = 7.3), 2.169 (1H, d, J = 15.1), 1.831−1.961 (1H, m), 1.387−1.743 (5H, m), 0.898 (9H, s), 0.885 (9H, s), −0.006−(0.096) (12H, m); ^{13}C NMR ($CDCl_3$) δ 177.6, 174.1, 136.5, 128.1, 84.9, 77.7, 72.6, 51.7, 50.8, 42.5, 41.9, 36.7, 34.0, 31.3, 25.6, 21.0, 18.2, −4.5, −5.0.

(3a*S*,4*R*,5*S*,6a*R*)-5-(*tert*-Butyl-dimethylsilyl)oxy-7-[5-(*tert*-butyldimethylsilyl)oxy-2-oxo-hexahydo-cyclopenta[*b*]furan-4-yl]-5-hydroxy-hept-6-enoic Acid Methyl Ester (43). To a stirred solution of **42** (0.033 g, 0.0776 mmol) in CH_2Cl_2 (1.0 mL) was

added Et_3N (0.109 mL, 0.776 mmol). After the mixture was stirred at room temperature for 5 min, TBDMSCl (58.48 mg, 0.388 mmol) and a catalytic amount of DMAP were added. The reaction mixture was stirred at reflux for 3.5 h. The reaction was allowed to cool to room temperature, after which a saturated solution of NH_4Cl (0.7 mL) was added. The two layers were separated. The aqueous layer was extracted with CH_2Cl_2 (3 × 0.5 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated under vacuum. The residue was purified by silica gel chromatography with CH_2Cl_2 as eluent to afford **43** (0.0347 g, 85%). ^{1}H NMR ($CDCl_3$) δ 5.765−5.881 (1H, dd, J = 15.5, 8.6), 5.513−5.604 (1H, dd, J = 15.5, 5.7), 5.090 (1H, t), 4.104−4.211 (2H, m), 3.676 (3H, s), 2.971−3.122 (1H, m), 2.829 (1H, dd, J = 18.3, 4.9), 2.388−2.569 (2H, m), 2.321 (2H, t, J = 7.3), 2.169 (1H, d, J = 15.1), 1.831−1.961 (1H, m), 1.387−1.743 (5H, m), 0.898 (9H, s), 0.885 (9H, s), −0.006−(0.096) (12H, m); ^{13}C NMR ($CDCl_3$) 177.8, 174.1, 137.1, 125.8, 84.9, 76.9, 72.7, 51.7, 51.0, 42.6, 42.4, 37.8, 34.2, 31.3, 26.1, 25.9, 20.7, 18.4, 18.2, −4.0, −4.5, −4.7, −4.9; HRMS calcd for $C_{23}H_{41}O_6Si_2$ [M − C_4H_9]$^+$, 469.2442; obsd, 469.2426; HRMS calcd for $C_{26}H_{47}O_6Si_2$ [M − CH_3]$^+$, 511.2914; obsd, 511.2911.

Methyl-7-{3α-hydroxy-5α-*tert*-butyldimethylsilyl)oxy)-2-(2-hydroxyethyl)-α-cyclopentane}-5(*S*)-*tert*-butyldimethylsilyl)oxy-hept-6-(*E*)-enoate (44). To a solution of **43** (0.034 g, 0.065 mmol) in MeOH/ether (0.375 mL/0.750 mL) was added $NaBH_4$ (37.83 mg, 0.517 mmol). Bubbling was observed. The reaction was stirred at room temperature for 35 min and then quenched with H_2O (0.5 mL). The aqueous layer was extracted with ethyl acetate (5 × 1.0 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated under vacuum. Purification of the residue by silica gel chromatography with 30% ethyl acetate in hexane as eluent afforded **44** (0.0193 g, 72.2%) and **45** (0.0062 g, 24.6%). Yields were calculated based on recovered starting material 55 (0.0075 g). ^{1}H NMR ($CDCl_3$) δ 5.734 (1H, dd, J = 15.3, 10.1), 5.429 (1H, J = 15.4, 5.6), 4.0701−4.308 (3H, m), 3.554−3.816 (5H, m), 2.545−2.673 (1H, m), 2.321 (2H, t, J = 7.3), 2.088−2.239 (1H, m), 1.421−1.74 (8H, m), 0.897 (18H, s), −0.029−(0.133) (12H, m); ^{13}C NMR ($CDCl_3$) 174.4, 136.2, 127.7, 77.6, 74.6, 72.8, 63.0, 51.7, 51.4, 45.9, 43.1, 38.2, 34.3, 31.3, 26.1, 20.9, 18.4, 18.3, −4.1, −4.5.

Methyl-7-{3α-triethylsilyl)oxy-5α-*tert*-butyldimethylsilyl)oxy)-2-(2-triethylsilyl)oxyethyl)-α-cyclopentane}-5(*S*)-*tert*-butyldimethylsilyl)oxy-hept-6-(*E*)-enoate (46). To a stirred solution of **44** (0.0193 mg, 0.0364 mmol) in CH_2Cl_2 (1.0 mL) was added Et_3N (0.0613 mL, 0.437 mmol). After the mixture was stirred at room temperature for 5 min, TESCl (49.0 μL, 0.219 mmol) and a catalytic amount of DMAP were added. The reaction mixture was stirred at reflux (56 °C) for 2.5 h and allowed to cool to room temperature, after which a saturated solution of NH_4Cl (1.0 mL) was added. Layers were separated. The aqueous layer was extracted with EtOAc (3 × 1.0 mL). The combined organic extracts were washed with H_2O, dried (Na_2SO_4), and concentrated under vacuum. The residue was purified by silica gel chromatography with 5% ethyl acetate in hexane as eluent to afford **46** (0.0233 g, 85%). ^{1}H NMR δ 5.860 (1H, dd, J = 15.3, 10.1), 5.415 (1H, dd, J = 15.6, 5.6), 4.132−4.247 (3H, m), 3.562−3.706 (5H, m), 2.419−2.627 (2H, m), 2.300 (2H, t, J = 7.3), 1.926−2.014 (1H, m), 1.460−1.777 (7H, m), 0.837−1.061 (36H, m), 0.483−0.681 (12H, m), 0.009−0.149 (12H, m); ^{13}C NMR (acetone-d_6) 173.9, 136.1, 129.4, 75.1, 74.0, 73.4, 62.4, 51.4, 45.1, 43.9, 38.8, 34.4, 26.5, 26.4, 21.6, 18.9, 18.7, 10.8, 10.4, 8.8, 8.6; HRMS calcd for $C_{39}H_{81}O_6Si_4$ [M]$^+$, 757.5110; obsd, 757.5111.

Methyl-7-{3α-triethylsilyl)oxy-5α-*tert*-butyldimethylsilyl)oxy)-2-ethanal-1α-cyclopentane}-5(*S*)-*tert*-butyldimethylsilyl)oxy-hept-6-(*E*)-enoate (47). At −70 °C, $(COCl)_2$ (0.027 mL, 2.0 M in CH_2Cl_2) was added to a solution of DMSO (8.34 μL, 0.1078 mmol) in CH_2Cl_2. After the mixture was stirred at −70 °C for 15 min, **46** (0.0082 mg, 0.011 mmol) in CH_2Cl_2 was added dropwise. The resulting solution was stirred at −70 °C for 20 min and allowed to warm to −50 °C and stirred for another 20 min. After the mixture was cooled to −70 °C, Et_3N (0.0263 mL, 0.188 mmol) was added. The solution was allowed to warm to room temperature and stirred for another hour. The mixture was diluted with H_2O (0.5 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 1.0 mL). The combined organic layer was dried (Na_2SO_4) and concentrated under vacuum. The residue was purified by flash chromatography to afford **47** (0.0056 mg, 81%). ^{1}H NMR (acetone-d_6) δ 9.759 (1H, s), 5.833 (1H, dd, J = 15.0, 8.9), 5.445 (1H, dd, J = 15.5, 5.5), 4.261−4.405 (1H, m), 4.113−4.250 (2H, m), 3.606 (3H, s), 2.386−2.931 (3H, m), 2.305 (2H, t, J = 7.3), 1.426−1.706 (7H, m), 0.781−1.036 (27H, m), 0.451−0.663 (6H, m), −0.030−(0.152) (12H, m). ^{13}C NMR (acetone-d_6) 202.7, 173.9, 136.6, 128.4, 74.7, 73.8, 73.3, 51.4, 50.7, 45.4, 43.5, 41.5, 38.6, 34.3, 26.4, 26.3, 21.5, 18.7, 10.6, 8.8.

Methyl-7-{3α-triethylsilyl)oxy-5α-*tert*-butyldimethylsilyl)oxy)-2α-[2,5-(*Z*,*Z*)-octadien]-1α-cyclopentane}-5(*S*)-*tert*-butyldimethylsilyl)oxy-hept-6-(*E*)-enoate (49). To a stirred solution of **48** (0.05 g, 0.106 mmol) in THF was added sodium hexamethyldisilazide (1.0 M, 95.4 μL) at −78 °C. The resulting deep orange reaction mixture was stirred at −78 °C for 10 min, after which **47** (0.008 g, 0.0125 mmol) and HMPA (8 μL) were added. The reaction was stirred at −78 °C for 20 min, allowed to warm to −40 °C, and was stirred at this temperature for another 30 min. The reaction was quenched with brine (1.0 mL). The aqueous layer was extracted with EtOAc (3× ∼1.5 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated under vacuum. Purification of the residue by silica gel chromatography afforded **49** (0.0073 g, 82%). ^{1}H NMR (acetone-d_6) δ 5.853 (1H, s), 5.221−5.500 (4H, m), 2.435−2.619 (2H, m), 2.010−2.350 (H, m), 1.810−1.889 (1H, m), 1.615−1.720 (4H, m), 1.485−1.585 (2H, m), 0.875−1.150 (27H, m), 0.630 (6H, m), 0.02−0.135 (12H, m); ^{1}H NMR ($CDCl_3$) 5.750 (1H, dd, J = 15.3, 10.1), 5.200−5.450 (5H, m), 4.010−4.120 (3H, m), 3.650 (3H, s), 2.720−2.810 (2H, m), 2.057−2.410 (8H, m), 1.450−1.750 (7H, m), 0.851−0.995 (27H, m), 0.545−0.625 (6H, m), 0.001−0.079 (12H, m); ^{13}C NMR (acetone-d_6) 173.9, 136.1, 132.2, 130.6, 129.3, 128.5, 128.3, 74.9, 74.1, 73.2, 51.6, 51.4, 48.8, 45.6, 38.8, 34.4, 26.5, 26.4, 26.4, 24.7, 21.6, 21.1, 20.4, 18.8, 18.8, 14.6, 10.4, 8.6.

7-{3α,5α-Dihydroxy-2α-[2,5-(*Z*,*Z*)-octadien]-1α-cyclopentane}-5(*S*)-hydroxy-hept-6-(*E*)-enoic Acid: 8,12-*iso*-iPF$_3$α-VI (10). To a stirred solution of **49** (0.0103 g, 0.0145 mmol) in THF (1.0 mL) was added 0.5 N HCl (0.580 mL). After being stirred at room temperature for 8 h, the reaction was quenched with a saturated solution of $NaHCO_3$ solution (pH was adjusted to ∼7). The aqueous layer was extracted with EtOAc (5× ∼1.0 mL). The combined organic extracts were washed with H_2O (2 × 1.5 mL), dried (Na_2SO_4), filtered, and concentrated under vacuum. At 0 °C, CH_2N_2 in ether was added dropwise to the crude product. The light yellow solution was allowed to stir at room temperature for 30 min, after which the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography to afford the ester derivative of **10** (0.004 g, 75%). ^{1}H NMR (acetone-d_6) δ 5.963 (1H, dd, J = 15.6, 10.4), 5.541−5.225 (5H, m), 4.222−3.995 (3H, m), 2.690−2.581 (1H, m), 1.979−2.390 (7H, m), 1.424−1.910 (7H, m), 0.947 (3H, t, J = 7.5); ^{13}C NMR (acetone-d_6) δ 174.1, 138.0, 132.1, 130.4, 129.7, 128.7, 128.4, 75.1, 72.8, 71.2, 51.6, 51.4, 48.3, 44.1, 37.8, 34.3, 26.2, 25.1, 21.9, 21.1, 14.6.

To the solution of the ester (0.004 g, 0.011 mmol) in ^{i}PrOH/H_2O (1.2:1, 0.500 mL) was added 395 μL of LiOH (0.1 M in ^{i}PrOH/H_2O), and the reaction was stirred at room temperature for 3 h. The reaction mixture was acidified with 0.5 N HCl and extracted with ethyl acetate (5 × 0.5 mL). The combined organic layer was washed with brine, dried (Na_2SO_4), and concentrated in a vacuum to give **10** (3.5 mg, 92% yield). ^{1}H NMR (acetone-d_6) δ 5.821 (1H, dd, J = 15.3, 10.5), 5.220−5.525 (5H, m), 4.150−4.195 (3H, m), 2.65 (1H, m), 2.010−2.30 (8H, m), 1.532−1.950 (7H, m), 0.985 (3H, t, J = 7.5); MS [M]$^+$ = 351; HRMS calcd for $C_{20}H_{32}O_5Na$ [M + Na]$^+$, 375.21436; obsd, 375.21420; HRMS calcd for

$C_{20}H_{32}O_5K$ $[M + K]^+$, 391.18842; obsd, 391.18813; HRMS calcd for $C_{20}H_{31}O_5$ $[M - H]^-$, 351.21674, obsd, 351.21770.

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Supporting Information Available: ^1H and ^{13}C NMR spectra of all compounds, NOESY of compound **33**, and HPLC tracing of compound **10**. Complete refs 7 and 8. This material is available free of charge via the Internet at http://pubs.acs.org.

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