Selective Synthesis of *ent*-15-*epi*-F_{2t}-Isoprostane and a Deuterated Derivative

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Dedicated to Professor Paul A. Wender on the occasion of his 60th birthday

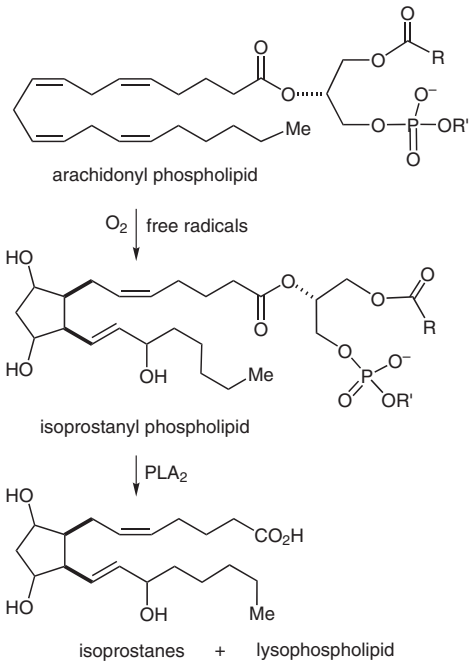
Abstract: Isoprostanes are an emerging class of lipid metabolites whose physiological properties are not well understood. The selective synthesis of *ent*-15-*epi*-F_{2t}-isoprostane, an isomer active in a preliminary screening assay is described. The synthesis features a regioselective cross-metathesis on an enantiomerically enriched divinyl cyclopentyl intermediate to selectively differentiate the side-chains of the target. The route provides the isoprostane, as well as a d_4-labeled analogue, in 14 steps from readily available starting materials.

Key words: asymmetric synthesis, metathesis, olefination, lipids

Isoprostanes are produced upon non-enzymatic, free radical peroxidation of phosphatidyl arachidoniates (Scheme 1).[1] In addition to possible activities within the lipid bilayer, hydrolytic release of these soluble lipid oxidation products may also lead to a variety of physiological responses. The biological activities of the isoprostanes that have been identified to date typically involve inflammatory responses, as well as activities related to smooth muscle growth factors,[2] and platelet aggregation factors.[3] These lipid metabolites are also recognized as important indicators of oxidative stress,[4] particularly in human maladies such as Alzheimer's disease,[5] diabetes,[6] and cancer.[7] While progress has been made in understanding the formation and distribution of the isoprostanes, significant questions remain regarding the physiological roles for these endogenous metabolites.

Total synthesis has begun to provide useful quantities of isoprostanes and derivatives required for their further study.[8] In this regard, we disclosed the preparation of all eight 15-F_2-isoprostane isomers via a stereodivergent strategy (Figure 1).[9] The key intermediate in the synthesis was obtained from a [2+2] photocycloaddition between a functionalized cyclopentenone and acetylene, followed by a ring-opening cross-metathesis.[10] Subsequent resolution of the racemic diastereomers by an asymmetric CBS-reduction provided the individual isoprostanes isomers in an enantiomerically enriched manner. This sequence offered simultaneous access to the complete library of 15-F_2-isoprostanes from a common synthetic precursor **1**.

Preliminary screening of the eight diastereomeric 15-F_2-isoprostanes in a whole blood platelet aggregation inhibition assay indicated that the unknown *ent*-15-*epi*-F_{2t}-isoprostane was more active than the known inhibitor, 15-F_{2t}-isoprostane.[11] To further study this particular isoprostane isomer, we modified our synthetic strategy toward the 15-F_2-isoprostanes to provide access to any specific isoprostane isomer. Herein, we describe how a regioselective olefin cross-metathesis of an enantiomerically enriched divinyl intermediate provides *ent*-15-*epi*-F_{2t}-isoprostane and a mass-labeled derivative in an enantioselective manner.



Scheme 1 Formation of the 15-F_2-isoprostanes

Our synthesis of *ent*-15-*epi*-F_{2t}-isoprostane begins with enone **4**, generated efficiently and selectively from an enzymatic desymmetrization of *meso*-diol **2** (with pancreatin lipase).[12,13] Enone **4** can be accessed by functional group manipulations from **3** in 80% overall yield (Scheme 2).[14]

A photochemical [2+2] cycloaddition between enone **4** and acetylene generates a mixture of bicyclo[3.2.0]heptenones **5** and **6** (**5**:**6** = 2:1, Scheme 3). DIBAL-H reduction of the mixture yields isomers **7**, **8**, **9**, and **10**, which are separable by silica gel chromatography. The reduction favors the desired hydroxyl stereochemistry by an 84:16 ratio. The major product **7** could be isolated and the remaining fractions could be oxidized with PCC back to a mixture of **5** and **6**. Preliminary results show that the un-

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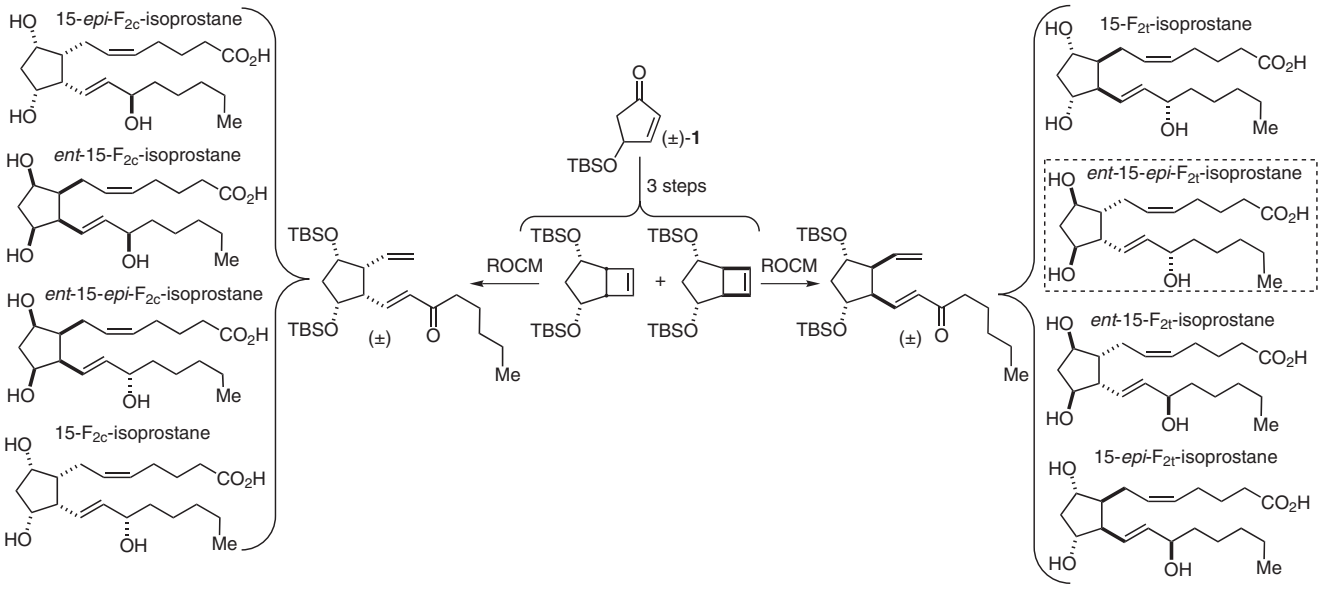
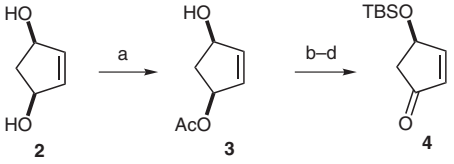
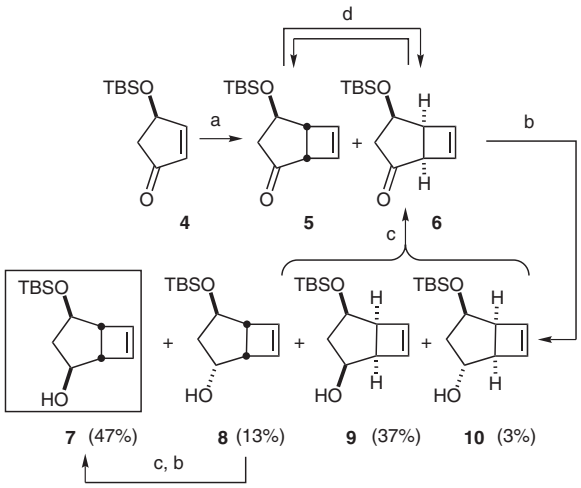


Figure 1 Stereodivergent synthesis of the 15-F_2-isoprostanes



Scheme 2 Preparation of optically enriched enone **4**. *Reagents and conditions*: (a) pancreatin lipase, vinyl acetate, THF, >99% ee, 55% yield after recryst.; (b) TBSCl, Et_3N, DMAP, CH_2Cl_2, 99%; (c) K_2CO_3, MeOH, 90%; (d) PCC, Celite, CH_2Cl_2, 90%.



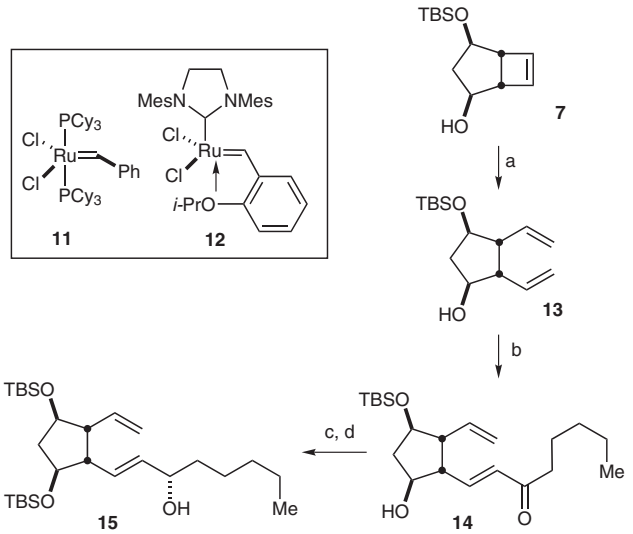
Scheme 3 Preparation of metathesis precursor **7**. *Reagents and conditions*: (a) acetylene, acetone, Pyrex, *hν*, 65% (86% conversion), **5/6** = 2:1; (b) DIBAL-H, toluene, –78 °C, 98%; (c) PCC, Celite, CH_2Cl_2, 98%; (d) *hν*, C_6H_6.

desired *syn-endo* photoadduct **6** can be photoisomerized using a Vycor filter to a 1:1 ratio of the desired *syn-exo* **5** isomer and the starting *syn-endo* **6**. This isomerization between the two diastereomers is likely occurring through a 1,3-acyl migration.[15] Given the reoxidation and isomerization pathways, in principle, all undesired isomers can be used to increase the overall yield of adduct **7**; however, in practice only cyclobutene **8** was routinely recycled.

Ring-opening cross-metathesis (ROCM) of *syn-exo* compound **7** in benzene using Grubbs' catalyst **11** under an ethylene atmosphere generates the divinyl species **13** in 87% yield (Scheme 4).[10,16] High dilution is required to avoid ring-opening metathesis polymerization (ROMP). In contrast, cross-metathesis (CM) of divinyl compound **13** with oct-1-en-3-one finds optimal conditions under higher concentration in CH_2Cl_2 at 40 °C with 5 mol% Hoveyda–Grubbs catalyst **12**. This leads to the desired mono-CM product in high selectivity; none of the other regioisomeric cross-metathesis product is observed and only trace amount of the double cross-metathesis product is obtained.[17] The newly formed enone in the desired product is formed exclusively with *E*-stereoselectivity. The high regioselectivity of the cross-metathesis could arise through ruthenium coordination by the free hydroxyl group on the cyclopentyl ring, although steric hindrance of the silyl protecting group on the other hydroxyl functionality may also play a role in dictating the selectivity of the reaction.

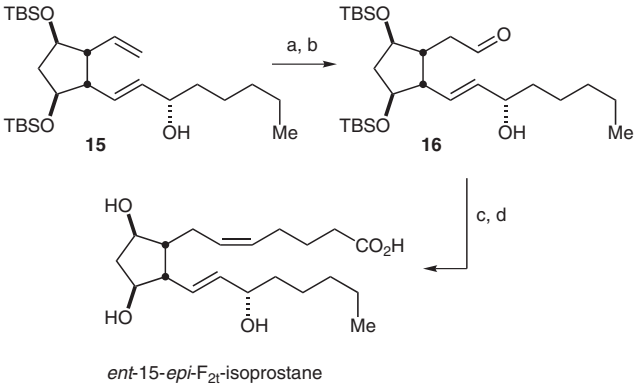
When cyclobutene **7** was subjected to a direct ROCM with oct-1-en-3-one using Hoveyda–Grubbs catalyst **12**, the desired compound **14** was not obtained; mainly ROMP of the cyclobutene was observed. Furthermore, Grubbs' catalyst **11** was unreactive in a ROCM with oct-1-en-3-one; only starting materials were recovered. Since the two metatheses require different reaction conditions, it was necessary to run these transformations in a stepwise fashion.

Silyl protection of the free hydroxyl group, followed by an asymmetric catalytic reduction of enone **14** with (*R*)-2-methyl-CBS-oxazaborolidine[18] and catecholborane led to alcohol **15** in high yield and diastereomeric excess (>95:5



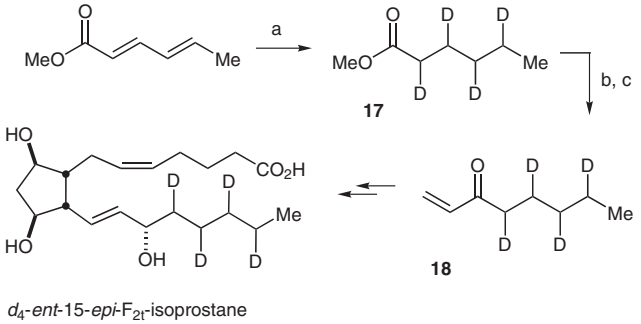
Scheme 4 Installation of the isoprostanyl side-chains through a regio- and stereoselective cross-metathesis. *Reagents and conditions*: (a) ethylene, 5 mol% **11**, benzene (0.01 M), 87%; (b) oct-1-en-3-one, 5 mol% **12**, CH_2Cl_2 (0.2 M), 40 °C, 70–90%; (c) TBSCl, Et_3N, DMAP, CH_2Cl_2, 99%; (d) (*R*)-2-methyl-CBS-oxazaborolidine, catecholborane, toluene, –78 °C; 99%.

dr) (Scheme 4).[19] Hydroboration of the terminal olefin with 9-BBN followed by an oxidative workup yields the primary alcohol, which was oxidized with catalytic TEMPO/NCS to generate aldehyde **16** (Scheme 5).[20] Wittig olefination forms the *Z*-olefin exclusively to complete the overall isoprostane structure. Removal of the two silyl protecting groups with TBAF delivered the desired *ent*-15-*epi*-F_{2t}-isoprostane in nearly quantitative yield. Of particular note, the efficiency of the synthetic route allows for sufficient quantity of *ent*-15-*epi*-F_{2t}-isoprostane to be prepared for further biological and chemical studies (i.e., > 0.1 g).



Scheme 5 Completion of *ent*-15-*epi*-F_{2t}-isoprostane. *Reagents and conditions*: (a) 9-BBN, THF; NaOH, H_2O_2, 80%; (b) TEMPO (cat.), NCS, *n*-Bu_4NCl, CH_2Cl_2/H_2O, 84% (85% conv.); (c) $[Ph_3P(CH_2)_4CO_2H]Br$, *t*-BuOK, 93%; (d) TBAF, THF, 99%.

In addition, this route also allowed for the preparation of a tetradeuterated analogue of *ent*-15-*epi*-F_{2t}-isoprostane.[21] Exhaustive reduction of methyl sorbate under D_2 gave **17** in 60% yield (Scheme 6). Weinreb amide[22] formation, followed by vinyl group addition provided tetradeuteroenone **18**. Enone **18** was then used for the cross-metathesis partner with **13** as described in Scheme 4. The rest of the synthesis was carried out in an analogous manner to that shown in Schemes 4 and 5 to yield d_4-*ent*-15-*epi*-F_{2t}-isoprostane.



Scheme 6 Synthesis of cross-metathesis partner **18** for the internal standard d_4-*ent*-15-*epi*-F_{2t}-isoprostane. *Reagents and conditions*: (a) D_2 (1 atm), Pd/C, MeOH, 60%; (b) MeNH(OMe)·HCl, *i*-PrMgCl, THF, –10 °C, 88%; (c) vinylmagnesium bromide, THF, 0 °C; 90%.

d_4-*ent*-15-*epi*-F_{2t}-Isoprostane was used as an internal standard in a GC/MS assay to detect isoprostanes in human urine.[23] Four diastereomeric 15-F_2-isoprsotanes were separated on an achiral GC column.[24] Although the known 15-F_{2t}-isoprostane was not detected in this assay, preliminary results revealed that the *ent*-15-*epi*-F_{2t}-isoprostane (or its enantiomer) was present in the urine sample.

In summary, we have achieved a stereoselective synthesis of *ent*-15-*epi*-F_{2t}-isoprostane through a regio- and stereoselective ring-opening cross-metathesis/cross-metathesis sequence. Enzymatic acylation generates the starting enone **4** as a single enantiomer. Photocycloaddition with acetylene followed by functional group manipulations provides an enantiomerically enriched precursor for the key metatheses. A catalyst-controlled asymmetric reduction completes one of the 15-F_2-isoprostanyl side-chains and allows for the installation of the second side-chain. The overall sequence provides efficient access to *ent*-15-*epi*-F_{2t}-isoprostane, as well as an isotopically labeled *ent*-15-*epi*-F_{2t}-isoprostane to be used for in vivo studies. Moreover, it is expected that the synthetic strategy can be readily modified to selectively access any of the 15-series isoprostanes. Further biological and chemical studies of *ent*-15-*epi*-F_{2t}-isoprostane are underway.

Starting materials and reagents purchased from commercial suppliers were used without further purification with the following exceptions: CH_2Cl_2, THF, benzene, Et_2O, and toluene were dried on alumina columns using a solvent dispensing system. *N*-Chlorosuccinimide was recrystallized from benzene/H_2O, and Bu_4NCl was recrystallized from acetone–Et_2O prior to use. 2,2,6,6-Tetramethyl-1-piperdinyloxy free radical (TEMPO) was purified by sublimation prior to use. The Hoveyda–Grubbs catalyst was purified according to published procedure.[25]

Oxygen- and/or moisture-sensitive reactions were carried out under N_2 in Schlenk glassware that was flame dried under high vacuum (~0.5 mmHg) and purged with N_2 prior to use. Air and/or moisture

sensitive solids were transferred in a glove box. Unless otherwise stated, reactions were stirred with a Teflon-covered stir bar. Concentration refers to the removal of solvent under reduced pressure using a Büchi rotary evaporator. Silica gel column chromatography was performed using Baxter brand silica gel 60 Å (230–400 mesh ASTM). Cerium ammonium molybdate was used as the TLC staining reagent. IR spectra (FTIR) were recorded on a Nicolet 210 FT-IR spectrometer, and reported in wave numbers (cm^{-1}). ^1H NMR and ^{13}C NMR spectra were measured on a Varian Gemini-400 instrument at 400 MHz (^1H NMR) and 100 MHz (^{13}C NMR). Chemical shifts are reported with the solvent as the internal standard [$CDCl_3$: δ = 7.26 (^1H NMR), δ = 77.0 (^{13}C NMR)]. Enantiomer ratio was determined by chiral GLC analysis (Supelco Betadex 120 column (30 m × 0.25 mm). Optical rotations were measured on a Rudolph Research Analytical Autopol IV polarimeter. High-resolution mass spectral (HRMS) analyses were performed on a Micromass LCT ESI-MS (positive mode) at the Mass Spectrometry Laboratory at Boston College.

4-(*tert*-Butyldimethylsilyloxy)cyclopent-2-enone (4)

Monoacetate compound **3** was obtained from literature procedure[26] after recrystallization with hexane–benzene as white needle-like crystals (55% yield, >99% ee). The optical purity was established by chiral GLC analysis. Acetate **3** (957 mg, 6.74 mmol) and DMAP (411 mg, 3.37 mmol) were dissolved in CH_2Cl_2 (67.0 mL) at 0 °C. Et_3N (2.80 mL, 20.2 mmol) and TBSCl (2.03 g, 13.5 mmol) were added to the mixture at 0 °C. The solution was warmed to 23 °C and stirred for 12 h. The mixture was diluted with CH_2Cl_2 (60 mL) and washed with aq 0.5 M HCl (2 × 40 mL), aq sat. $NaHCO_3$ (1 × 50 mL) and brine (1 × 50 mL). The organic layer was dried ($MgSO_4$), filtered, and solvent was removed under reduced pressure to give TBS-protected cyclopentenyl acetate **I** (not shown) as a colorless oil, which was pure by ^1H NMR spectroscopy (1.71 g, 99% yield). An aqueous solution of 1 M K_2CO_3 (6.37 mL, 6.37 mmol) was added to a stirred solution of **I** (1.63 g, 6.37 mmol) in MeOH (27.0 mL) and was stirred for 3 h at 23 °C. The mixture was diluted with CH_2Cl_2 (20 mL) and washed with brine (2 × 50 mL). The aqueous layer was back-extracted with CH_2Cl_2 (3 × 25 mL) and the combined organic layers were dried ($MgSO_4$) and filtered. The solvent was removed under reduced pressure and the residue was purified by silica gel chromatography (hexanes–EtOAc, 3:1) to give hydroxy cyclopentenyl compound **II** (not shown) as a colorless oil (1.30 g, 96% yield). PCC (1.96 g, 9.11 mmol) and Celite (2.90 g) were stirred in CH_2Cl_2 (63.0 mL) at 23 °C. Intermediate **II** (1.30 g, 6.07 mmol) in CH_2Cl_2 (5.0 mL) was added to the mixture. After 2 h, the solution was diluted with Et_2O (15 mL) and was filtered through a pad of Celite (4 cm thick) over silica gel (4 cm thick) and washed with hexanes–Et_2O (1:1, 50 mL). Solvent was removed under reduced pressure and the residue was purified by silica gel chromatography (hexanes–Et_2O, 5:1) to give enone **4** as a colorless oil (1.18 g, 92%); $[\alpha]_D^{20}$ +43.5 (*c* 1.08, $CHCl_3$).

IR (film): 2954 (m), 2928 (m), 2887 (w), 2857 (m), 1742 (s), 1471 (w), 1354 (m), 1182 (w), 1109 (m), 1071 (m), 900 (m), 838 (m), 777 cm^{-1} (m).

^1H NMR ($CDCl_3$, 400 MHz): δ = 7.50 (1 H, dd, *J* = 6.0, 2.4 Hz), 6.23 (1 H, dd, *J* = 5.6, 1.2 Hz), 5.04–5.02 (1 H, m), 2.75 (1 H, dd, *J* = 18.0, 5.6 Hz), 2.29 (1 H, dd, *J* = 18.0, 2.0 Hz), 0.957 (9 H, s), 0.17 (6 H, s).

^{13}C NMR ($CDCl_3$, 100 MHz): δ = 206.3, 163.8, 134.5, 71.1, 45.3, 26.1, 18.5, –4.27, –4.30.

HRMS (ESI^+): *m*/*z* calcd for $C_{11}H_{20}O_2Si$ + Na (M + Na): 235.1130; found: 235.1126.

4-(*tert*-Butyldimethylsilyloxy)bicyclo[3.2.0]hept-6-en-2-ones (5, 6)

In a 3.0 L-photochemical vessel, enone **4** (2.40 g, 11.3 mmol) was dissolved in acetone (2.80 L). A Pyrex immersion well containing a Pyrex sleeve (~3.5 mm thickness) was placed into the vessel and the solution was sparged with acetylene for 5 min. A 100 W medium-pressure mercury vapor lamp was placed in the immersion well and the stirred solution was irradiated with constant bubbling of acetylene for a total of 42 h (reaction progress monitored by GC). Solvent was removed under reduced pressure and the residue was purified by silica gel chromatography (hexanes–Et_2O, gradient 20:1, 10:1, 5:1) to give a mixture of **5** and **6** [**5**:**6** = 2:1, 86% conv., 1.73 g, 74% yield, based on recovered starting material (BRSM)]. The ^1H NMR and ^{13}C NMR were identical to published results.[9]

4-(*tert*-Butyldimethylsilyloxy)bicyclo[3.2.0]hept-6-en-2-ol (7)

In a flame-dried Schlenk flask, a mixture of **5** and **6** (1.00 g, 4.20 mmol) was dissolved in toluene (17.4 mL) and cooled to 78 °C. DIBAL-H (823 μL, 4.62 mmol) was added to the mixture and stirred for 3 h, after which the reaction was quenched carefully with MeOH (3.0 mL). The solution was warmed to 23 °C and diluted with Et_2O (10 mL). The reaction was opened to air and aq sat. solution of Rochelle's salt (sodium potassium tartrate, 20 mL) was added to the mixture and stirred for 1 h. The solution was extracted with Et_2O (2 × 30 mL), and the combined organic layers were washed with aq 1 M HCl (2 × 30 mL) and brine (1 × 30 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated. The residue was purified by silica gel chromatography (hexanes–Et_2O; gradient, 10:1, 5:1, 3:1) to obtain **7**, **8**, **9**, **10** (467 mg, 47%; 129 mg, 13%; 367 mg, 37%; 30 mg, 3% respectively; total yield: 993 mg, 98% yield). Data shown only for cyclobutene **7**; $[\alpha]_D^{20}$ –0.74 (*c* = 0.27, $CHCl_3$).

IR (film): 3530 (b), 3040 (w), 2953 (s), 2929 (s), 2894 (m), 2855 (m), 1469 (m), 1408 (m), 1253 (m), 1088 (s), 1053 (s), 1002 (s), 835 (s), 773 (s), 697 cm^{-1} (m).

^1H NMR ($CDCl_3$, 400 MHz): δ = 6.05 (1 H, d, *J* = 2.8 Hz), 5.99, (1 H, d, *J* = 2.8 Hz), 4.18 (1 H, d, *J* = 3.6 Hz), 4.00 (1 H, dd, *J* = 11.2, 4.4 Hz), 3.36 (1 H, s), 3.23, (1 H, s), 3.10 (1 H, d, *J* = 11.2 Hz), 2.31 (1 H, dt, *J* = 14.0, 4.4 Hz), 1.87 (1 H, d, *J* = 14.4 Hz), 0.89 (9 H, s), 0.09 (6 H, s).

^{13}C NMR ($CDCl_3$, 100 MHz): δ = 140.3, 138.7, 73.8, 72.9, 57.2, 56.5, 40.4, 26.2, 18.4, –4.38, –4.50.

HRMS (ESI^+): *m*/*z* calcd for $C_{13}H_{24}O_2Si$ + Na (M + Na): 263.1443; found: 263.1445.

4-(*tert*-Butyldimethylsilyloxy)-2,3-divinylcyclopentanol (13)

Grubbs catalyst **11** (74.0 mg, 89.8 μmol) was stirred in benzene (87.9 mL) and sparged with ethylene (balloon) for 5 min. Cyclobutene **7** (432 mg, 1.80 mmol) in benzene (2.0 mL) was added to the mixture and stirred under an ethylene atmosphere (balloon) at 23 °C for 15 h. The mixture was opened to air and ethyl vinyl ether (1.0 mL) was added and stirred for 5 min. Silica gel (4.20 g) was then added and stirred for another 15 min. The slurry was filtered through a plug of silica gel and washed with hexanes–EtOAc (3:1, 30 mL). The filtrate was concentrated and purified by silica gel chromatography (hexanes–Et_2O, 5:1) to obtain **13** as a clear yellow oil (426 mg, 88%); $[\alpha]_D^{20}$ +1.6 (*c* 0.37, $CHCl_3$).

IR (film): 3359 (b), 2955 (s), 2928 (s), 2857 (s), 1639 (w), 1471 (m), 1256 (s), 1089 (b), 912 (s), 836 (s), 775 cm^{-1} (s).

^1H NMR ($CDCl_3$, 400 MHz): δ = 5.66 (1 H, ddd, *J* = 17.1, 10.2, 8.8 Hz), 5.60–5.51 (1 H, m), 5.14–5.06 (3 H, m), 5.03 (1 H, dd, *J* = 6.0, 1.7 Hz), 4.06–4.01 (2 H, m), 2.84 (1 H, ddd, *J* = 8.5, 8.3, 5.4 Hz), 2.77 (1 H, ddd, *J* = 8.8, 8.3, 3.1 Hz), 2.36 (1 H, dt, *J* = 14.1, 6.5 Hz), 2.00 (1 H, d, *J* = 6.7 Hz), 1.69 (1 H, dt, *J* = 14.1, 4.0 Hz), 0.88 (9 H, s), 0.05 (6 H, s).

^{13}C NMR ($CDCl_3$, 100 MHz): δ = 137.3, 137.0, 116.8, 116.7, 77.4, 76.7, 56.6, 55.7, 43.4, 26.2, 18.4, –4.1, –4.3.

HRMS (ESI$^+$): *m*/*z* calcd for $C_{15}H_{28}O_2Si$ + Na (M + Na): 291.1756; found: 291.1753.

1-[3-(*tert*-Butyldimethylsilyloxy)-5-hydroxy-2-vinylcyclopentyl]oct-1-en-3-one (14)

Divinyl compound **13** (425 mg, 1.58 mmol) and oct-1-en-3-one (300 mg, 2.37 mmol) were dissolved in CH_2Cl_2 (8.0 mL). Hoveyda–Grubbs catalyst **12** (50.0 mg, 79.0 μmol) was added and the mixture was heated to 40 °C. The mixture was stirred for 5 h, then cooled to 23 °C and opened to air. Ethyl vinyl ether (2.0 mL) was added and the mixture was stirred for 5 min. The solvent was removed under reduced pressure and the residue was purified by silica gel chromatography (hexanes–EtOAc, 5:1) to obtain **14** as a light brown oil (429 mg, 74%[27]); $[\alpha]_D^{20}$ –11 (*c* 0.30, $CHCl_3$).

IR (film): 3425 (b), 2956 (s), 2928 (s), 2857 (s), 1669 (m), 1625 (m), 1459 (m), 1359 (m), 1248 (m), 839 cm^{-1} (s).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 6.67 (1 H, dd, *J* = 15.8, 9.0 Hz), 6.19 (1 H, dd, *J* = 15.8, 1.1 Hz), 5.49 (1 H, ddd, *J* = 16.4, 10.8, 9.6 Hz), 5.09–5.04 (2 H, m), 4.13 (1 H, tt, *J* = 7.3, 4.7 Hz), 4.08 (1 H, dt, *J* = 5.9, 3.1 Hz), 3.01 (1 H, td, *J* = 8.3, 5.5 Hz), 2.88–2.83 (1 H, m), 2.50 (2 H, t, *J* = 7.3 Hz), 2.39 (1 H, ddd, *J* = 14.3, 7.2, 5.7 Hz), 2.12 (1 H, d, *J* = 7.5 Hz), 1.74 (1 H, dt, *J* = 13.8, 3.3 Hz), 1.64–1.56 (2 H, m), 1.35–1.24 (4 H, m), 0.91–0.87 (3 H, m), 0.89 (9 H, s), 0.06 (6 H, s).

^{13}C NMR ($CDCl_3$, 100 MHz): δ = 200.3, 145.1, 135.9, 131.3, 117.8, 77.3, 76.6, 57.1, 54.2, 43.6, 41.0, 31.8, 26.2, 24.3, 22.8, 18.4, 14.3, –4.1, –4.2.

HRMS (ESI$^+$): *m*/*z* calcd for $C_{21}H_{38}O_3Si$ + Na (M + Na): 389.2488; found: 389.2489.

1-[3,5-Bis(*tert*-butyldimethylsilyloxy)-2-vinylcyclopentyl]oct-1-en-3-ol (15)

Enone **14** (362 mg, 0.99 mmol) and DMAP (60.2 mg, 0.49 mmol) were dissolved in CH_2Cl_2 (9.9 mL). Et_3N (412 μL, 2.96 mmol) and TBSCl (297 mg, 1.97 mmol) were added to the mixture. The solution was stirred at 23 °C for 12 h. The mixture was diluted with CH_2Cl_2 (40 mL) and washed with 0.5 M HCl (2 × 15 mL), aq sat. $NaHCO_3$ (1 × 20 mL), and brine (1 × 20 mL). The organic layer was dried ($MgSO_4$), filtered, and solvent was removed under reduced pressure. The residue was purified by silica gel chromatography (hexanes–Et_2O, 15:1) to obtain bis(TBS-protected) enone **III** (not shown) as a colorless oil (435 mg, 92%). To a flame-dried Schlenk flask, intermediate **III** (300 mg, 0.62 mmol) and toluene (3.12 mL) were added. The solution was cooled to –78 °C and (*R*)-methyl-CBS-oxazoborilidine catalyst (1 M in toluene, 312 μL, 0.31 mmol) was added followed by catecholborane (266 μL, 2.50 mmol). The mixture was stirred at –78 °C for 15 h. MeOH (2.0 mL) was added to quench the reaction and the mixture was gradually warmed to 23 °C. The solution was diluted with EtOAc (20 mL) and the combined organic layers were washed with aq 1 M NaOH (1 × 20 mL), aq 1 M HCl (1 × 20 mL), and brine (1 × 20 mL). The organic layer was dried ($MgSO_4$), filtered, and solvent was removed under reduce pressure. The residue was purified by silica gel chromatography (hexanes–Et_2O, 8:1) to afford **15** as a colorless oil (303 mg, 99%); $[\alpha]_D^{20}$ +1.9 (*c* 1.3, $CHCl_3$).

IR (film): 3342 (b), 3070 (w), 2955 (s), 2925 (s), 2853 (s), 1638 (w), 1469 (m), 1361 (m), 1258 (s), 1095 (b), 835 (s), 775 cm^{-1} (s).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.63–5.41 (3 H, m), 5.06–5.04 (1 H, m), 5.03–5.01 (1 H, m), 4.05 (1 H, qd, *J* = 10.2, 6.5 Hz), 3.95–3.89 (2 H, m), 2.74–2.65 (2 H, m), 2.36 (1 H, dt, *J* = 14.0, 7.1 Hz), 1.59 (1 H, dt, *J* = 13.7, 5.6 Hz), 1.54–1.43 (2 H, m), 1.36–1.25 (7 H, m), 0.91–0.86 (3 H, m), 0.87 (18 H, s), 0.018 (6 H, s), 0.013 (3 H, s), 0.007 (3 H, s).

^{13}C NMR ($CDCl_3$, 100 MHz): δ = 135.5, 129.5, 76.2, 72.9, 61.6, 53.5, 46.0, 44.4, 37.4, 32.4, 31.8, 25.9, 25.2, 22.7, 18.2, 18.1, 14.2, –3.9, –4.4, –4.5.

Diastereomeric ratio was determined by comparison of the ^{1}H NMR spectrum (C_6D_6) with the authentic 1:1 diastereomeric mixture (estimated detection limits of 20:1).

***ent*-15-*epi*-F_{2t}-Isoprostane**

9-BBN (0.5 M in THF, 1.0 mL, 0.50 mmol) was added to neat **15** (111 mg, 0.23 mmol) at 0 °C under N_2 and stirred for 5 min. The mixture was warmed to 23 °C and stirred for 5.5 h. The reaction was opened to air and diluted with EtOAc (1.5 mL). The solution was cooled to 0 °C and aq 3 M NaOH (545 μL, 1.64 mmol) and 30% H_2O_2 (449 μL, 4.67 mmol) were added. After 1 h, the mixture was extracted with EtOAc (3 × 5 mL) and the combined organic layers were washed with aq sat. $NaHCO_3$ (2 × 10 mL) and brine (1 × 10 mL). The organic layer was dried ($MgSO_4$), filtered, and solvent was removed under reduced pressure. The residue was purified by silica gel chromatography (hexanes–EtOAc, gradient 3:1, 1:1) to obtain 1-[3,5-bis(*tert*-butyldimethylsilyloxy)-2-(2-hydroxyethyl)cyclopentyl]oct-1-en-3-ol (**IV**; not shown) as a colorless oil (96.4 mg, 85%); $[\alpha]_D^{20}$ –15.6 (*c* 2.40, $CHCl_3$).

IR (film): 3388 (b), 2956 (s), 2925 (s), 2857 (s), 1647 (m), 1468 (m), 1363 (m), 1258 (m), 1061 (m), 851 (m), 770 (m), 666 cm^{-1} (m).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.51 (1 H, dd, *J* = 15.3, 6.5 Hz), 5.37 (1 H, dd, *J* = 15.4, 9.9 Hz), 4.06 (1 H, q, *J* = 6.4 Hz), 3.90–3.84 (2 H, m), 3.72–3.60 (2 H, m), 2.56–2.51 (1 H, m), 2.35 (1 H, dt, *J* = 13.7, 6.9 Hz), 2.24 (1 H, dt, *J* = 14.7, 8.1 Hz), 1.67–1.44 (6 H, m), 1.39–1.24 (7 H, m), 0.91–0.89 (2 H, m), 0.89 (9 H, s), 0.88–0.86 (1 H, m), 0.87 (9 H, s), 0.07 (6 H, s), 0.02 (6 H, s).

^{13}C NMR ($CDCl_3$, 100 MHz): δ = 135.5, 129.5, 76.7, 76.2, 72.9, 61.7, 53.6, 46.0, 44.4, 37.4, 32.4, 31.9, 25.9, 25.2, 22.7, 18.2, 18.1, 14.2, –3.9, –4.4, –4.5.

HRMS (ESI$^+$): *m*/*z* calcd for $C_{27}H_{56}O_4Si_2$+ Na (M + Na): 523.3615; found: 523.3625.

Diol **IV** (232 mg, 0.463 mmol) and Bu_4NCl (25.7 mg, 92.6 μmol) were stirred in CH_2Cl_2 (3.8 mL) and H_2O (carbonate buffer, pH 8.6, 3.8 mL) at 23 °C. NCS (68.0 mg, 0.509 mmol) and TEMPO (14.5 mg, 92.6 μmol) was added to the mixture and stirred for 3 h. The mixture was diluted with CH_2Cl_2 (10 mL) and the CH_2Cl_2 layer washed with brine (2 × 15 mL). The aqueous layer was back-extracted with CH_2Cl_2 (3 × 15 mL) and the combined organic layers were dried ($MgSO_4$), filtered, and concentrated. The residue was purified by silica gel chromatography (hexanes–EtOAc, 4:1) to obtain the aldehyde **16** as a thick orange oil (184 mg, 99%, BSRM) and starting material (49 mg, 80% conv.). *t*-BuOK (329 mg, 2.94 mmol) in THF (4.9 mL) was added to a stirred solution of carboxybutyltriphenylphosphonium bromide (651 mg, 1.47 mmol) in THF (3.1 mL) at 23 °C. After this bright orange ylide solution was stirred for 20 min, it was syringed into a stirred solution of the aldehyde (183 mg, 0.37 mmol) in THF (1.1 mL). After the reaction was stirred for 3.5 h, aq sat. NH_4Cl (15 mL) and glacial AcOH (1 mL) were added. The solution was extracted with EtOAc (3 × 10 mL), dried ($MgSO_4$), filtered, and concentrated. The residue was purified by silica gel chromatography (hexanes–EtOAc–AcOH, 5:1:0.1) to obtain TBS-protected-isoprostane **V** (not shown) as a colorless oil (200 mg, 93%). TBAF (1 M in THF, 2.69 mL, 2.69 mmol) was added to neat **V** (196 mg, 0.37 mmol) at 23 °C and stirred overnight. The mixture was opened to air and diluted with EtOAc (5.0 mL) and washed with aq sat. NH_4Cl (2 × 5 mL) solution. The aqueous layer was back-extracted with EtOAc (3 × 10 mL) and the combined organic layers were washed with brine (1 × 25 mL). The solution was dried ($MgSO_4$), filtered, and solvent was removed under reduced pressure. The residue was purified by silica gel chromatography (EtOAc–MeOH–AcOH, 20:1:0.1) to obtain *ent*-15-*epi*-F_{2t}-isopros-

tane as a pale yellow oil (104 mg, 87%); $[\alpha]_D^{20}$ –1.1 (*c* 0.55, MeOH).

IR (film): 3363 (b), 2999 (w), 2923 (m), 2847 (m), 1698 (s), 1405 (w), 1235 (w), 1052 (m), 965 cm^{-1} (m).

^1H NMR ($CDCl_3$, 400 MHz): δ = 5.57 (1 H, dd, *J* = 15.3, 6.6 Hz), 5.52–5.40 (3 H, m), 4.12 (1 H, q, *J* = 6.4 Hz), 4.04–4.00 (2 H, m), 3.94–3.52 (3 H, br s), 2.78 (1 H, dq, *J* = 8.6, 3.9 Hz), 2.44 (1 H, dt, *J* = 14.7, 6.9 Hz), 2.32 (2 H, t, *J* = 6.4 Hz), 2.21–2.04 (4 H, m), 1.99–1.90 (1 H, m), 1.74–1.61 (3 H, m), 1.60–1.45 (2 H, m), 1.39–1.25 (6 H, m), 0.88 (3 H, t, *J* = 6.5 Hz).

^{13}C NMR ($CDCl_3$, 100 MHz): δ = 176.5, 135.2, 129.7, 129.4, 129.2, 76.5, 76.4, 73.1, 53.8, 50.8, 42.3, 37.3, 32.6, 31.8, 27.0, 26.3, 25.2, 24.4, 22.7, 14.2.

HRMS (ESI^+): *m*/*z* calcd for $C_{20}H_{34}O_5$ + Na (M + Na): 377.2304; found: 377.2290.

***d*$_4$-*ent*-15-*epi*-F$_{2t}$-Isoprostane**

The synthesis was carried out analogously as above, starting with cross-metathesis of the appropriate d_4-oct-1-en-3-one and divinyl cyclopentane **13**;[28] $[\alpha]_D^{20}$ –0.6 (*c* 0.33, MeOH).

IR (film): 3420 (b), 2930 (m), 2873 (m), 1709 (s), 1413 (w), 1255 (w), 1193 (w), 1060 (m), 966 cm^{-1} (w).

^1H NMR ($CDCl_3$, 400 MHz): δ = 5.56 (1 H, dd, *J* = 15.2, 6.8 Hz), 5.48–5.40 (3 H, m), 4.18 (1 H, br s), 4.14 (1 H, q, *J* = 6.0 Hz), 4.00–4.01 (2 H, m), 2.81–2.76 (1 H, m), 2.43 (1 H, dt, *J* = 14.8, 6.8 Hz), 2.32 (2 H, t, *J* = 6.5 Hz), 2.21–2.04 (4 H, m), 1.98–1.93 (1 H, m), 1.70–1.61 (3 H, m), 1.54–1.46 (2 H, m), 1.34–1.24 (4 H, m), 0.89–0.84 (3 H, m).

^{13}C NMR ($CDCl_3$, 100 MHz): δ = 175.8, 135.6, 130.1, 129.7, 129.4, 76.6, 76.5, 73.2, 53.8, 50.9, 42.4, 32.7, 27.0, 26.4, 26.4, 24.5, 14.1.

HRMS (ESI^+): *m*/*z* calcd for $C_{20}H_{30}D_4O_5$ + Na (M + Na): 381.2550; found: 381.2555.

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