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Synthesis of the four enantiomerically-pure isomers of 15-F2t-isoprostane

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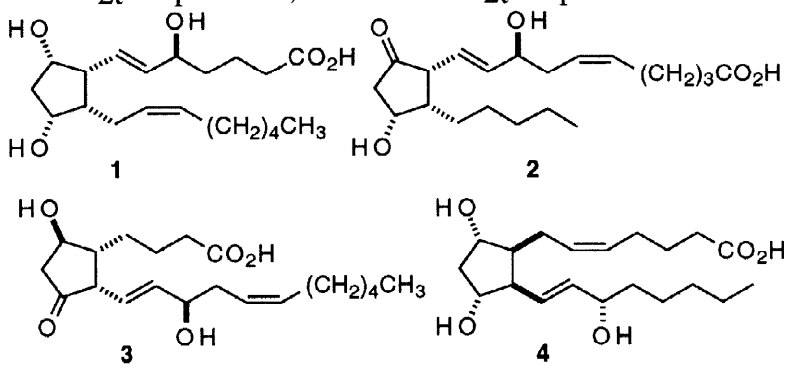
Abstract

Syntheses of the four enantiomerically-pure isomers of 15-F2t-isoprostane are described. The key step is the lipase-mediated resolution of a pseudo-meso diol, to give the regioisomeric acetates in high enantiomeric purity. Improved procedures for the preparation of the pseudo-meso diol are also reported.

Keywords: Chromatography; Eicosanoids; Enzymes and enzyme reactions; Prostanoids.

Introduction

In 1990, Roberts and co-workers reported that a series of prostaglandin-like compounds are produced *in vivo* in humans independent of the cyclooxygenase enzymes, by free radical-mediated oxidation of membrane-bound arachidonic acid [1]. These oxidation products have been named the isoprostanes. Interestingly, levels of F2-isoprostanes in normal human biological fluids exceed levels of prostaglandins derived *via* cyclooxygenase by at least an order of magnitude. In addition to F-ring isoprostanes, it was recently reported that E-ring and D-ring isoprostanes are also produced in abundance *in vivo*. There are ninety-six isoprostanes, divided into four groups, depending on whether hydroxylation has occurred at C-5, C-8, C-12, or C-15. An individual member of the group is named according to its substitution, following a modification [2] of prostaglandin nomenclature. Thus **1** is 5-F2c-isoprostane, **2** is 8-E2c-isoprostane, **3** is *ent*-12-D2t-isoprostane, and **4** is 15-F2t-isoprostane.



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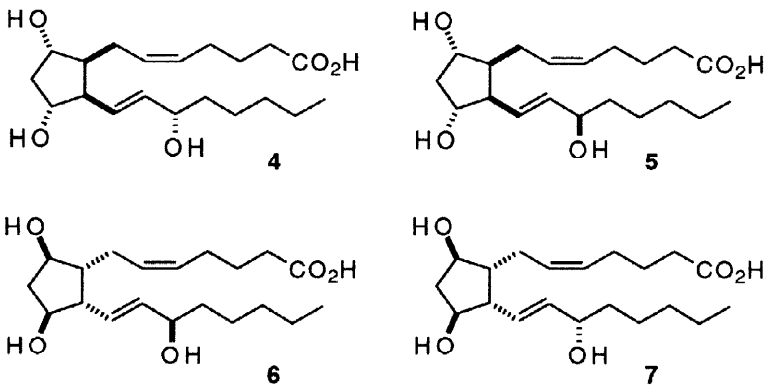
While the detailed physiological investigation of these compounds has just begun [3], it has already been shown that the kidney failure and death associated with severe liver disease is a consequence of the production and release of the isoprostanes [4]. It has also been demonstrated that the effects of 15-F_{2t}-isoprostane (**4**) on the renal vasculature result from specific receptor binding [3a, 3b, 5]. To investigate the physiological activity of the isoprostanes, it will be necessary to prepare each of these by chemical synthesis [6,7.8]. We report the first preparation of each of the four enantiomerically-pure isomers of 15-F_{2t}-isprostane (**4** – **7**).



Results and Discussion

We recently reported the diasteroselective synthesis of (±)-15-F_{2t}-isoprostane ethyl ester (**8**) (Scheme 1) [6c]. The key feature of our synthesis was the aldol condensation of diazo ketone **10**, readily prepared from the benzoyl ketone **9**, with the commercially available (*E*, *E*)-decadienal **11**. Cyclization of the resulting silylated aldol product **12**, followed by kinetic opening of the cyclopropane ring of the bicyclic ketone **13** with thiophenol and BF_3•OEt_2 gave **14a** and **14b** (*ca.* 9 : 1). Reduction and Mislow rearrangement then gave (±)-15-F_{2t}-isprostane ethyl ester (**8**). This strategy allowed control not just of ring functionality and relative configuration, but also control of the relative configuration of the secondary allylic hydroxy substituent on the pendant side chain.

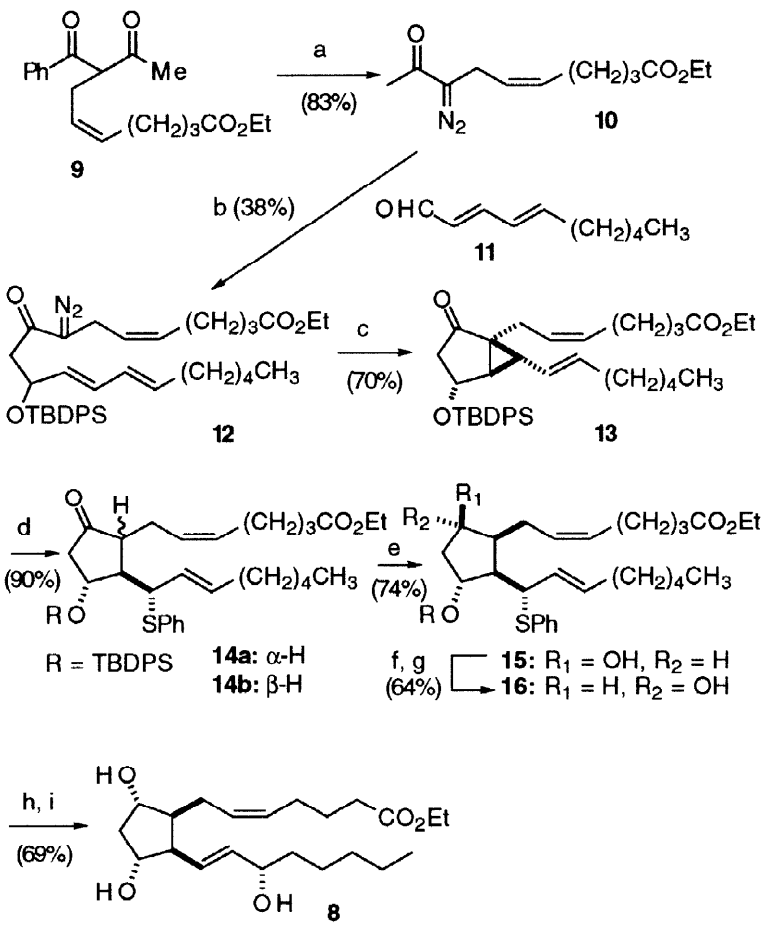
The isoprostanes are produced *in vivo* as racemic mixtures of C-15 diastereomers. Rather than design a specific synthesis of each of the four enantiomerically-pure isomers of a particular isoprostane when all four are needed for screening, it seemed more sensible to develop a stereodivergent synthesis that would lead to each of the four from common intermediates.

In order to prepare larger quantities of particular isoprostanes in enantiomerically-pure form, we needed to first improve the overall yield of Scheme 1. Our investigation began with the preparation of diazoketone **10** from diketone **9** [6c, 10]. We observed that the yield of this reaction decreased as the scale increased. After some investigation, we found that the concentration of the reaction was critical. If the concentration was kept below 0.05 M, the diazo transfer reaction proceeded in a reproducibly good yield.

We next investigated the critical aldol condensation of the diazoketone **10** with (*E*,*E*)-decadienal **11** (Scheme 2). We had previously reported[6c] that treatment of the diazoketone **10** with KHMDS and the LiBr-coordinated aldehyde **11** in THF, followed by protection of the

resulting aldol **18** with t-butyldiphenylsilyl chloride proceeded in 38% overall yield. We have subsequently observed that this procedure does not work consistently. After some exploration, we have found that exposure of the diazoketone **10** to KHMDS in toluene followed by addition of a mixed solution of triethylchlorosilane (TESCl) and aldehyde **11** in toluene at -78°C gave

Scheme 1[a]



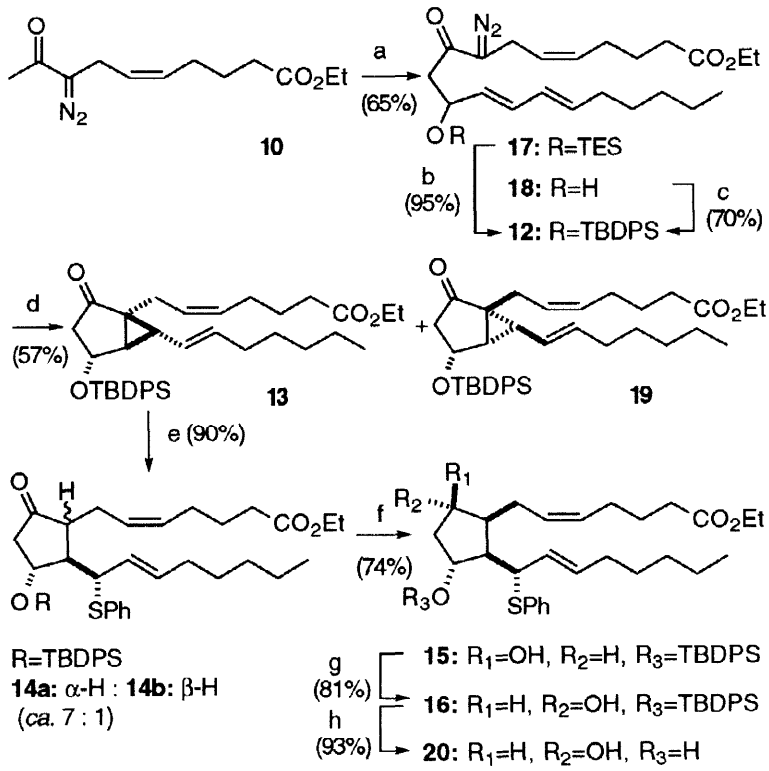
[a]*Reagents and conditions*: (a) *p*-NBSA, DBU, CH_2Cl_2, 0°C; (b) KHMDS, **11**, LiBr, THF, -78°C; TBDPSCl, imidazole, 4-DMAP, CH_2Cl_2, rt; (c) $Rh_2(oct)_4$, CH_2Cl_2, rt; (d) PhSH, BF_3•OEt_2, CH_2Cl_2, -78°C; (e) $NaBH_4$, MeOH, 0°C; (f) *p*-nitrobenzoic acid, Ph_3P, DEAD, PhH, rt; (g) K_2CO_3, EtOH, rt; (h) mCPBA, CH_2Cl_2, -78°C; $(MeO)_3P$, EtOH, -78°C ~ rt; (i) n-Bu_4NF, THF, rt.

the TES-protected aldol **17** together with a small amount of the free aldol **18** in 65% combined yield. This reaction did *not* proceed well in THF. Control experiments suggest that TESCl is acting not only as a trapping agent for the initially formed potassium alkoxide, but also as a "super proton" facilitating the initial addition.[11] As the TES group of **17** could not survive under the conditions for cyclopropane ring opening with thiophenol and BF_3•OEt_2, we then effected protecting group exchange to give **12**. Cyclization of **12** with rhodium(II) octanoate proceeded with 3.5 : 1 diastereoselectivity, as we had reported, to provide the bicyclic ketones

13 and **19** in 57% combined yield. The balance of the material appears to be the unstable tetraene resulting from β-hydride elimination.

Cyclopropane ring opening with thiophenol and BF_3•OEt_2 in CH_2Cl_2 at -78°C gave the ketones **14a** and **14b** as an inseparable mixture (*ca.* 7 : 1) in 90% yield. Reduction of this mixture produced alcohols **15** and **16** in 42% and 32% yields, respectively, accompanied by a minor amount of the reduction product from **14b**. As Mitsunobu coupling of **15** did not proceed efficiently, the undesired β-alcohol **15** was oxidized by the Dess-Martin reagent [12], then again reduced with $NaBH_4$ to give the same mixture of **15** and **16**. Desilylation of the α-alcohol **16** with n-Bu_4NF in THF afforded the racemic diol **20**.

Scheme 2[a]

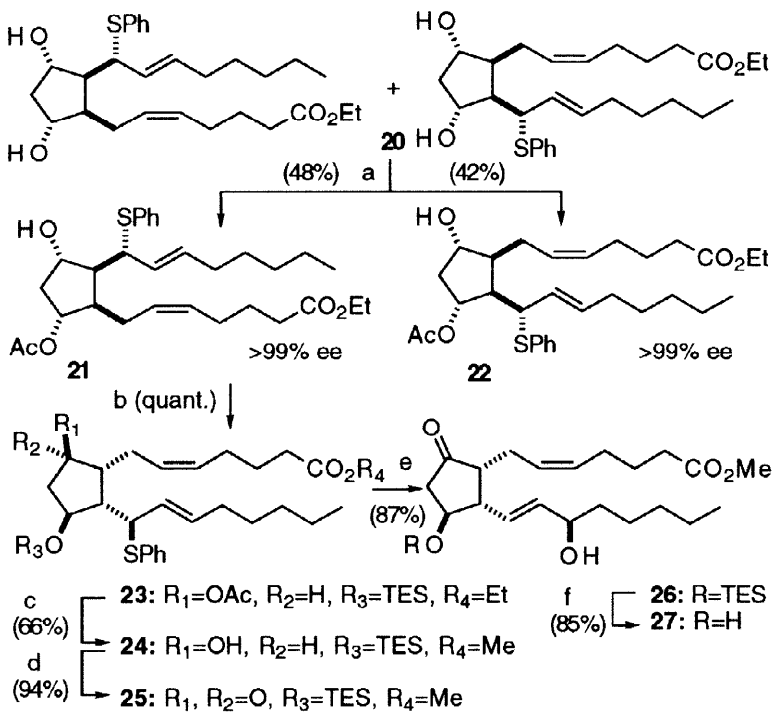


[a]*Reagents and conditions*: (a) KHMDS, toluene, -78°C; **11**, TESCl, toluene, -78°C (**17** : **18** = 3.7 : 1); (b) n-Bu_4NF, NH_4Cl (solid), THF, 0°C; TBDPSCl, imidazole, 4-DMAP, CH_2Cl_2, rt; (c) TBDPSCl, imidazole, 4-DMAP, CH_2Cl_2, rt; (d) $Rh_2(oct)_4$, Ch_2Cl_2, rt (**13** : **19** = 3.5 : 1); (e) PhSH, BF_3•OEt_2, CH_2Cl_2, -78 ~ -20°C (*cis* : *trans* = 7 : 1); (f) $NaBH_4$, MeOH, 0°C; (g) Dess-Martin periodinate, CH_2Cl_2, rt; $NaBH_4$, MeOH, 0°C; (h) n-Bu_4NF, THF, rt.

Because of the pseudosymmetry of the two enantiomers of the racemic **20** (Scheme 3), we thought that it might be possible to effect enzymatic resolution [13]. We screened three lipases, Amano AK, AY, and PS, in three solvents, vinyl acetate, THF, and diisopropyl ether, using vinyl acetate as the acylating agent. We found that the most efficient combination was Amano lipase AK (*Pseudomonas sp.* immobilized on Celite) in neat vinyl acetate, for 5 days at room temperature. This furnished the mono-acetates **21** and **22** in 48% and 42% yields, each with

>99% ee (determined by HPLC analysis with a CHIRACEL OD HPLCcolumn. The absolute configuration of these acetates was unambiguously determined by conversion of the 9-acetate **21** into (–)-8-*epi*-prostaglandin E_2 methyl ester (*ent*-15-E_{2t}-isoprostane methyl ester [2]) (**27**). Thus, silylation of the alcohol **21** gave **23**, which on sequential ethanolysis and methanolysis yielded the methyl ester **24**. Oxidation of **24** with the Dess-Martin periodinane [12] gave ketone **25**. Subsequent sulfur oxidation and Mislow rearrangement [14] converted **25** to the allylic alcohol **26**. Finally, desilylation with 52% aqueous HF in pyridine furnished *ent*-15-E_{2t}-isoprostane methyl ester (**27**). The physicochemical properties of **27** were identical with those of 15-E_{2t}-isoprostane methyl ester [8] except for the sign of specific optical rotation {$[\alpha]^{20}_D$ = -62.0 (*c* 0.075, MeOH), lit.[8] $[\alpha]_D$ = +40.95 (*c* 0.075, MeOH)}.

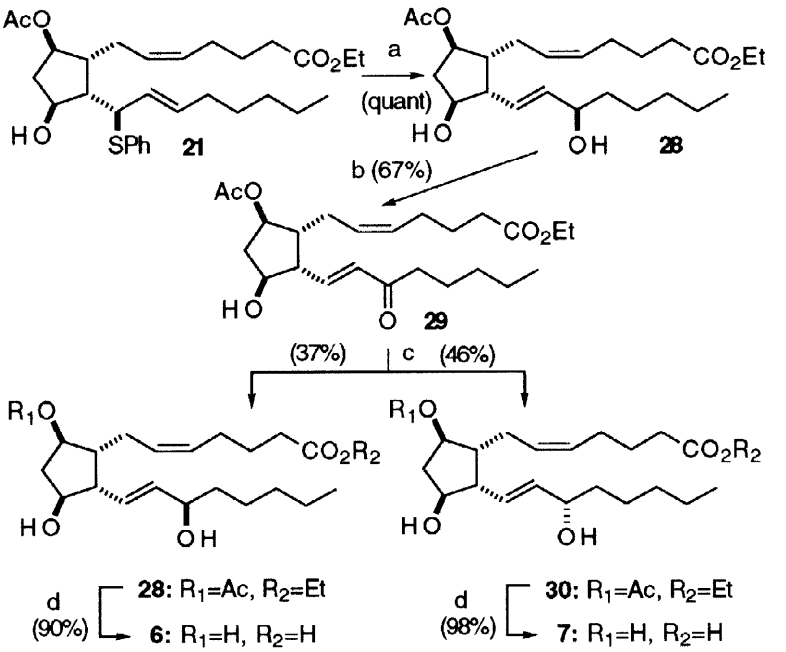
Scheme 3[a]

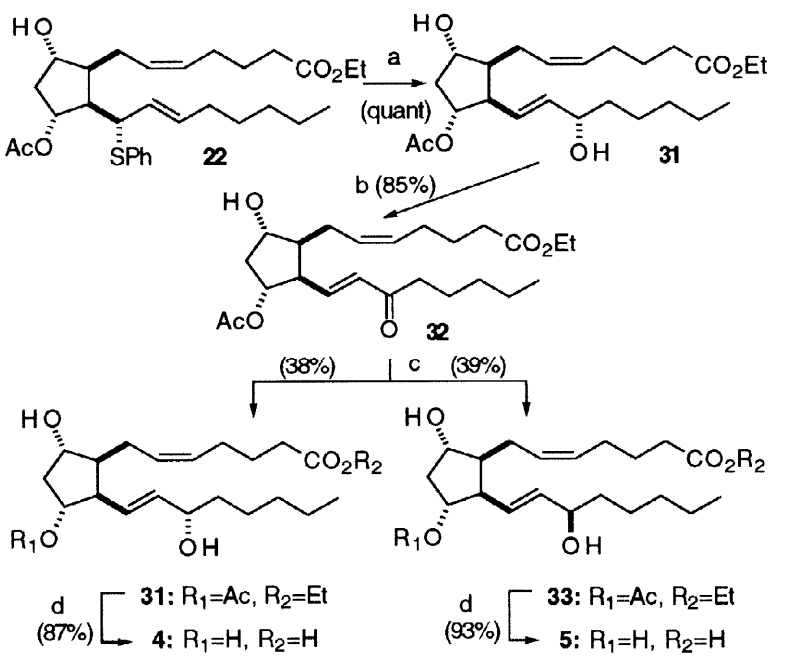


[a]*Reagents and conditions*: (a) Amano lipase AK, vinyl acetate, rt; (b) TESCl, imidazole, 4-DMAP, CH_2Cl_2, 0°C ~ rt; (c) K_2CO_3, EtOH, 65°C; K_2CO_3, MeOH, 65°C; (d) Dess-Martin periodinate, CH_2Cl_2, rt; (e) mCPBA, CH_2Cl_2, -78°C; $(MeO)_3P$, MeOH, -78°C ~ rt; (f) 52% aq. HF, Py, CH_3CN, rt.

With the requisite enantiomerically-pure acetates **21** and **22** in hand, we embarked on the synthesis of the four enantiomerically-pure isomers of 15-F_{2t}-isoprostane (**4** – **7**), as shown in Scheme 4. Oxidation and Mislow rearrangement [14] of the 9-acetate **21** gave the allylic alcohol **28**, which on treatment with DDQ [6c,15] in 1,4-dioxane-CH_2Cl_2 (1 : 1) gave the enone **29** in 67% overall yield. Reduction of the enone **29** with $NaBH_4$ produced the epimeric allylic alcohols **28** and **30** in 37% and 46% yields, respectively. These were separately hydrolyzed with LiOH in THF-H_2O (1 : 1) to furnish *ent*-15-F_{2t}-isoprostane (**6**) and its 15-epimer **7** in 90%

Scheme 4[a]





[a]*Reagents and conditions*: (a) mCPBA, CH_2Cl_2, -78°C; $(MeO)_3P$, EtOH, -78°C ~ rt; (b) DDQ, CH_2Cl_2-1,4-dioxane (1 : 1), 40°C; (c) $NaBH_4$, MeOH, 0°C ~ rt; (d) $LiOH{\bullet}H_2O$, THF-H_2O (1 : 1), rt.

and 98% yields, respectively. The enantiomeric 15-F_{2t}-isoprostane (**4**) and its 15-epimer **5** were also prepared from the 11-acetate **22** following the same procedure.

Conclusion

We have developed a practical synthesis of the four enantiomerically-pure isomers of 15-F_{2t}-isoprostane (**4** – **7**) using an enzymatic resolution of the pseudo-meso diol **20** as the key step. This synthesis will make **4** - **7** available in sufficient quantity to allow the detailed assessment of their physiological activity.

Experimental [16]

Ethyl (*Z*)-8-Diazo-9-oxo-dec-5-en-1-oate (10). To a stirred solution of the diketone **9** (7.0 g, 22.2 mmol) in CH_2Cl_2 (260 mL) at 0°C was added DBU (3.98 mL, 26.6 mmol). After 5 min, a solution of *p*-nitrobenzenesulfonyl azide (5.56 g, 24.4 mmol) in CH_2Cl_2 (230 mL) was added dropwise over 15 min. After an additional 1 h, the reaction mixture was partitioned between CH_2Cl_2 and, sequentially, saturated aqueous $NaHCO_3$ and brine. The combined organic extract was dried (Na_2SO_4) and concentrated. The residue was chromatographed to afford the diazoketone **10** (3.96 g, 75%) as a pale yellow oil; TLC Rf (EtOAc / petroleum ether = 2 / 8) = 0.37. This compound was identical with the material we had previously reported [6c].

Ethyl (5*Z*,12*E*,14*E*)-11-(Triethylsilyloxy)-8-diazo-9-oxo-5,12,14-eicosatrienoate (17) and Ethyl (5*Z*,12*E*,14*E*)-8-Diazo-11-hydroxy-9-oxo-5,12,14-eicosatrienoate (18). To a stirred solution of the diazoketone **10** (1.65 g, 6.93 mmol) in toluene (140 mL) at -78°C was added dropwise a 0.5M toluene solution of KHMDS (14.6 mL, 7.30 mmol) over 15 min. After 5 min, a solution of (*E*, *E*)-decadienal **11** (1.27 g, 8.31 mmol) and TESCl (1.40 mL, 8.31 mmol) in toluene (30 mL) was added. After an additional 15 min, the reaction mixture was partitioned between EtOAc and, sequentially, saturated aqueous NH_4Cl and brine. The combined organic extract was dried (Na_2SO_4) and concentrated. The residue was chromatographed to afford the TES-protected aldol **17** (1.68 g, 48%) as a pale yellow oil; TLC Rf (EtOAc / petroleum ether = 2 / 8) = 0.74; ^1H NMR δ 6.15 (dd, 1H, *J* = 10.3 and 15.0 Hz), 5.97 (dd, 1H, *J* = 10.3 and 15.0 Hz), 5.35 - 5.75 (m, 4H), 4.58 - 4.73 (m, 1H), 4.12 (q, 2H, *J* = 7.1 Hz), 3.08 (d, 2H, *J* = 7.4 Hz), 2.73 (dd, 1H, *J* = 8.4 and 13.5 Hz), 2.46 (dd, 1H, *J* = 4.4 and 13.5 Hz), 2.30 (t, 2H, *J* = 7.4 Hz), 2.02 - 2.15 (m, 4H), 1.69 (m, 2H), 1.23 - 1.42 (m, 6H), 1.25 (t, 3H, *J* = 7.1 Hz), 0.92 (t, 9H, *J* = 7.2 Hz), 0.88 (t, 3H, *J* = 7.1 Hz), 0.56 (q, 6H, *J* = 7.9 Hz); ^{13}C NMR δ up: 191.4, 173.3, 68.5, 60.3, 47.0, 33.6, 32.6, 31.4, 28.8, 26.4, 24.7, 22.5, 20.2, 4.8; down: 135.6, 132.8, 132.5, 130.4, 129.1, 123.7, 71.2, 14.2, 14.0, 6.8; IR (film) 2956, 2073, 1737, 1633, 1459, 1372, 1241, 1177, 990, 744 cm^{-1}; EI MS *m/z* (rel intensity) 476 ($M^+ - N_2$, 59), 447 (91), 401 (25), 344 (60), 271 (16), 229 (41), 189 (32), 161 (37), 115 (100); HRMS calcd for $C_{28}H_{48}O_4Si$ 476.3322, found 476.3317. This was followed by the recovered diazoketone **10** (112 mg, 7%). Further elution gave the free aldol **18** (335 mg, 13%) as a pale yellow oil; TLC Rf (EtOAc / petroleum ether = 2 / 8) = 0.30; ^1H NMR δ 6.25 (dd, 1H, *J* = 10.4 and 15.2 Hz), 6.01 (dd, 1H, *J* = 10.4 and 15.2 Hz), 5.72 (dt, 1H, *J* = 7.4 and 15.2 Hz), 5.55 - 5.63 (m, 2H), 5.40 (m, 1H), 4.64 (q, 1H, *J* = 6.0 Hz), 4.13 (q, 2H, *J* = 7.1 Hz), 3.2 - 3.4 (br s, 1H), 3.10 (d, 2H, *J* = 7.4 Hz), 2.67 (d, 1H, *J* = 6.0 Hz), 2.66 (m, 1H), 2.30 (t, 2H, *J* = 7.4 Hz), 2.04 - 2.14 (m, 4H), 1.69 (quint, 2H, *J* = 7.4 Hz), 1.3 - 1.4 (m, 6H), 1.26 (t, 3H, *J* = 7.1 Hz), 0.88 (t, 3H, *J* = 7.1 Hz); ^{13}C NMR δ up: 192.7, 173.4,

68.1, 60.3, 44.3, 33.5, 32.6, 31.3, 28.8, 26.5, 24.6, 22.5, 19.9; down: 136.2, 133.3, 131.3, 130.3, 129.1, 123.3, 69.0, 14.2, 14.0; IR (film) 3448, 2927, 2074, 1733, 1626, 1373, 1176, 990 cm^{-1}; FAB MS *m/z* (rel intensity) 413 (M^+ + Na, 30), 307 (18), 195 (15), 154 (100); FAB HRMS calcd for $C_{22}H_{34}O_4N_2Na$ 413.2416, found 413.2405.

Ethyl (5*Z*,12*E*,14*E*)-11-[(*tert*-Butyldiphenylsilyl)oxy]-8-diazo-9-oxo-5,12,14-eicosatrienoate (12) from TES-ether 17. To a stirred solution of the TES-ether **17** (1.03 g, 2.04 mmol) in THF (20 mL) at 0°C were added solid NH_4Cl (547 mg, 10.2 mmol) followed by a 1M THF solution of n-Bu_4NF (2.25 mL, 2.25 mmol). After additional 20 min, the reaction mixture was partitioned between CH_2Cl_2 and, sequentially, saturated aqueous NH_4Cl and brine. The organic extract was dried (Na_2SO_4) and concentrated. This residue was dissolved in CH_2Cl_2 (30 mL). To this solution at rt were added imidazole (417 mg, 6.13 mmol), 4-DMAP (50 mg, 0.41 mmol), and t-butylchlorodiphenylsilane (1.33 mL, 5.11 mmol). After an additional 24 h, the reaction mixture was partitioned between CH_2Cl_2 and, sequentially, saturated aqueous NH_4Cl and brine. The organic extract was dried (Na_2SO_4) and concentrated. The residue was chromatographed to afford the t-butyldiphenylsilyl ether **12** (1.22 g, 95%) as a pale yellow oil; TLC R_f (EtOAc / petroleum ether = 2 / 8) = 0.73. This compound was identical with the material we had previously reported [6c].

Ethyl (5*Z*,12*E*,14*E*)-11-[(*tert*-Butyldiphenylsilyl)oxy]-8-diazo-9-oxo-5,12,14-eicosatrienoate (12) from the free aldol 18. To a stirred solution of the free aldol **18** (550 mg, 1.41 mmol) in CH_2Cl_2 (10 mL) were added imidazole (288 mg, 4.23 mmol), 4-DMAP (35 mg, 0.28 mmol), and t-butylchlorodiphenylsilane (0.92 mL, 3.53 mmol). After an additional 28 h, the reaction mixture was partitioned between CH_2Cl_2 and, sequentially, saturated aqueous NH_4Cl and brine. The organic extract was dried (Na_2SO_4) and concentrated. The residue was chromatographed to afford the t-butyldiphenylsilyl ether **12** (620 mg, 70%) as a pale yellow oil.

Bicyclic Ketones 13 and 19. To a stirred solution of $Rh_2(oct)_4$ (8 mg, 0.11 mmol) in CH_2Cl_2 (5 mL) at rt was added dropwise over 1 h a solution of the diazoketone (650 mg, 1.04 mmol) in CH_2Cl_2 (15 mL). After an additional 10 min, the reaction mixture was concentrated. The residue was chromatographed to afford the bicyclic ketone **19** (78 mg, 13%) as a colorless oil; TLC R_f (petroleum ether / methyl t-butyl ether (MTBE) / CH_2Cl_2 = 90 / 6 / 4) = 0.34. This was followed by the bicyclic ketone **13** (272 mg, 44%) as a colorless oil; TLC R_f (petroleum ether / MTBE / CH_2Cl_2 = 90 / 6 / 4) = 0.33. These compounds were identical with the materials we had previously reported [6c].

Ethyl (5*Z*, 8*S, 11*R**, 12*R**, 13*S**, 14*E*)-11-[(*tert*-Butyldiphenylsilyl)oxy]-9-oxo-13-(phenylthio) prosta-5,14-dienoate (14a) and Ethyl (5*Z*,8*R**,11*R**,12*R**,13*S**,14*E*)-11-[(*tert*-Butyldiphenylsilyl)oxy]-9-oxo-13-(phenylthio)prosta-5,14-dienoate (14b).** To a stirred solution of the bicyclic ketone **13** (350 mg, 0.58 mmol) and thiophenol (0.12 mL, 1.17 mmol) in CH_2Cl_2 (12 mL) at -78°C was added BF_3•OEt_2 (0.18 mL, 1.46 mmol). After 10 min, the mixture was warmed to -20°C. After an additional 1 h, the reaction mixture was partitioned between CH_2Cl_2 and, sequentially, saturated aqueous $NaHCO_3$ and brine. The organic extract was dried (Na_2SO_4) and concentrated. The residue was chromatographed to afford as an inseparable *ca.* 7 : 1 mixture of the thioether **14a** and its 8-epimer **14b** (370 mg, 90%) as a colorless oil; TLC R_f (petroleum ether / MTBE / CH_2Cl_2 = 88 / 8 / 4) = 0.39. These compounds were identical with the materials we had previously reported [6c].

Ethyl (5*Z*, 8*S, 9*R**, 11*R**, 12*R**, 13*S**, 14*E*)-11-[(*tert*-Butyldiphenylsilyl)oxy]-9-hydroxy-13-(phenylthio)prosta-5,14-dienoate (15) and Ethyl (5*Z*,8*S**,9*S**,11*R**,12*R**, 13*S**,14*E*)-11-[(*tert*-Butyldiphenylsilyl)oxy]-9-hydroxy-13-(phenylthio)prosta-5,14-dienoate (16).** To a stirred solution of the inseparable ketone epimers **14a** and **14b** (*ca.* 7 : 1) (365 mg, 0.51 mmol) in MeOH (6 mL) at 0°C was added $NaBH_4$. After an additional 1 h, the reaction mixtute was partitioned between EtOAc and, sequentially, saturated aqueous NH_4Cl and brine. The organic extract was dried (Na_2SO_4) and concentrated. The residue was chromatographed to afford the β-alcohol **15** (153 mg, 42%) as a colorless oil; TLC Rf (petroleum ether / MTBE / CH_2Cl_2 = 88 / 8 / 4) = 0.24. This was followed by the α-alcohol **16** (115 mg, 32%) as a colorless oil; TLC Rf (petroleum ether / MTBE / CH_2Cl_2 = 88 / 8 / 4) = 0.14. These compounds were identical with the materials we had previously reported [6c].

Conversion of β-alcohol 15 to α-alcohol 16. To a stirred solution of the β-alcohol **15** (20 mg, 0.028 mmol) in CH_2Cl_2 (1 mL) was added Dess-Martin periodinane (14.3 mg, 0.034 mmol). After an additional 30 min, the reaction was cooled in an ice bath. The resulting precipitate was filtered and washed with Et_2O. Evaporation of the combined filtrate gave a residue that was dissolved in MeOH (1 mL). To this solution at 0°C was added $NaBH_4$ (8.3 mg, 0.22 mmol). After an additional 1 h, the reaction mixture was partitioned between EtOAc and, sequentially, saturated aqueous NH_4Cl and brine. The organic extract was dried (Na_2SO_4) and concentrated. The residue was chromatographed to affford the β-alcohol **15** (11.6 mg, 58%) as a colorless oil. Further elution gave the α-alcohol **16** (6.8 mg, 81% from **15**) as a colorless oil.

Ethyl (5*Z*, 8*S, 9*S**, 11*R**, 12*R**, 13*S**, 14*E*)-9,11-dihydroxy-13-(phenylthio)prosta-5,14-dienoate (20).** To a stirred solution of the silyl ether **16** (73 mg, 0.10 mmol) in THF (1.5 mL) at 0°C was added a 1M THF solution of n-Bu_4NF (0.3 mL, 0.31 mmol). After an additional 24 h, the reaction mixture was partitioned between EtOAc and, sequentially, saturated aqueous NH_4Cl and brine. The organic extract was dried (Na_2SO_4) and concentrated. The residue was chromatographed to afford the diol **20** (45 mg, 93%) as a colorless oil; TLC Rf (petroleum ether / MTBE = 3 / 7) = 0.2; ^1H NMR δ 7.42 - 7.46 (m, 2H), 7.27 - 7.33 (m, 3H), 5.24 - 5.41 (m, 3H), 5.13 (dt, 1H, *J* = 6.8 and 15.3 Hz), 4.38 (ddd, 1H, *J* = 3.5, 6.8, and 12.5 Hz), 4.11 (q, 2H, *J* = 7.1 Hz), 4.05 (br s, 1H), 3.52 (dd, 1H, *J* = 9.4 and 11.6 Hz), 2.98 (d, 1H, *J* = 2.9 Hz), 2.47 - 2.57 (m, 2H), 2.27 (t, 2H, *J* = 7.4 Hz), 2.0 - 2.11 (m, 2H), 1.89 (q, 2H, *J* = 6.8 Hz), 1.55 - 1.72 (m, 5H), 1.25 (t, 3H, *J* = 7.1 Hz), 1.07 - 1.28 (m, 6H), 0.85 (t, 3H, *J* = 7.1 Hz); ^{13}C NMR δ up: 173.5, 133.3, 60.3, 41.9, 33.6, 32.1, 31.1, 28.9, 26.7, 24.7, 24.3, 22.4; down: 134.4, 132.3, 10.2, 129.2, 128.7, 128.4, 127.8, 76.2, 74.4, 54.6, 52.3, 50.6, 14.2, 14.0; IR (film) 3399, 2927, 1733, 1438, 1174, 968, 692 cm^{-1}; FAB MS *m/z* (rel intensity) 497 (M^+ + Na, 100), 365 (34), 330 (26), 329 (92), 303 (28), 301 (32), 293 (28), 281 (28), 257 (56), 221 (41), 219 (47), 207 (41), 205 (26), 199 (30); FAB HRMS calcd for $C_{28}H_{42}O_4SNa$ 497.2702, found 497.2735.

Lipase catalyzed resolution of the racemic diol 20. To a stirred solution of the racemic diol **20** (160 mg, 0.34 mmol) in vinyl acetate (6.75 mL) was added Amano lipase AK (800 mg, 5 mass eq.). After 5 days, the insoluble material was filtered and the filtrate was concentrated. The residue was chromatographed to afford the 9-acetate **21** (84 mg, 48%) as a colorless oil; TLC Rf (petroleum ether / MTBE = 3 / 7) = 0.53; ^1H NMR δ 7.43 - 7.46 (m, 2H), 7.28 - 7.34 (m, 3H), 5.38 - 5.45 (m, 1H), 5.27 - 5.33 (m, 2H), 5.11 (dt, 1H, *J* = 6.8 and 15.3 Hz), 4.89 (d, 1H, *J* = 6.2 Hz), 4.39 (dt, 1H, *J* = 4.2 and 9.2 Hz), 4.11 (q, 2H, *J* = 7.1 Hz), 3.52 (dd, 1H, *J* = 9.6

and 11.7 Hz), 3.06 (br s, 1H), 2.64 (ddd, 1H, J = 6.2, 9.2, and 16.0 Hz), 2.33 - 2.40 (m, 1H), 2.27 (t, 2H, J = 7.5 Hz), 2.08 - 2.13 (m, 2H), 2.06 (s, 3H), 1.99 (q, 2H, J = 7.2 Hz), 1.89 (q, 2H, J = 6.8 Hz), 1.78 (m, 1H), 1.62 - 1.69 (m, 3H), 1.25 (t, 3H, J = 7.1 Hz), 1.0 - 1.26 (m, 6H), 0.85 (t, 3H, J = 7.1 Hz); ^{13}C NMR δ up: 173.5, 170.6, 133.0, 60.3, 39.2, 33.6, 32.0, 31.1, 28.9, 26.7, 24.7, 23.9, 22.4; down: 134.5, 132.6, 130.6, 128.9, 128.8, 128.1, 127.7, 76.9, 75.9, 54.7, 52.3, 47.8, 21.5, 14.2, 14.0; IR (film) 3490, 2927, 1733, 1245, 1024, 692 cm^{-1}; FAB MS *m/z* (rel intensity) 539 (M^+ + Na, 10), 329 (43), 281 (30), 221 (31), 206 (36), 147 (100), 136 (26), 133 (32), 132 (48), 117 (20), 105 (27); FAB HRMS calcd for $C_{30}H_{44}O_5SNa$ 539.2807, found 539.2797. $[\alpha]^{20}_D$ -8.51 (*c* 0.51, $CHCl_3$). The ee of the 9-acetate **21** was determined to be >99% by HPLC with a CHIRALCEL OD column (Daicel Chemical Industry Ltd.) using hexane-2-propanol (9 : 1, v/v) as a mobil phase. Acetate **21** had a retention time of 7.8 min, and its enantiomer had a retention time of 14.0 min. Further elution gave the 11-acetate **22** (73 mg, 42%) as a colorless oil; TLC R_f (petroleum ether / MTBE = 3 / 7) = 0.48; ^{1}H NMR δ 7.35 - 7.38 (m, 2H), 7.21 - 7.28 (m, 3H), 5.23 - 5.43 (m, 4H), 4.98 (dt, 1H, J = 6.8 and 15.1 Hz), 4.12 (q, 2H, J = 7.1 Hz), 4.12 (br s, 1H), 3.53 (t, 1H, J = 10.2 Hz), 2.77 (dt, 1H, J = 7.0 and 10.2 Hz), 2.61 (ddd, 1H, J = 5.6, 9.1, and 15.8 Hz), 2.28 (t, 2H, J = 7.5 Hz), 2.10 (s, 3H), 2.05 - 2.18 (m, 2H), 2.00 (q, 2H, J = 7.2 Hz), 1.83 (q, 2H, J = 6.8 Hz), 1.60 - 1.73 (m, 5H), 1.26 (t, 3H, J = 7.1 Hz), 1.02 - 1.29 (m, 6H), 0.84 (t, 3H, J = 7.1 Hz); ^{13}C NMR δ up: 173.5, 171.1, 134.3, 60.3, 40.6, 33.7, 32.0, 31.1, 28.9, 26.7, 24.7, 24.5, 22.4; down: 134.4, 132.4, 130.5, 129.0, 128.5, 128.2, 127.5, 77.0, 74.6, 53.5, 50.1, 48.7, 21.6, 14.2, 14.0; IR (film) 3450, 2928, 1735, 1369, 1246, 1025, 968, 692; FAB MS *m/z* (rel intesity) 539 (M^+ + Na, 20), 347 (23), 329 (100), 303 (19), 301 (19), 257 (43); FAB HRMS calcd for $C_{30}H_{44}O_5SNa$ 539.2807, found 539.2817. $[\alpha]^{20}_D$ -48.6 (*c* 0.49, $CHCl_3$). The ee of the 11-acetate **22** was determined to be >99% by HPLC with a CHIRALCEL OD column (Daicel Chemical Industry Ltd.) using hexane-2-propanol (99 : 1, v/v) as a mobil phase. Acetate **22** had a retention time of 63.7 min and its enantiomer had a retention time of 70.4 min.

Ethyl (5*Z*, 8*R*, 9*R*, 11*S*, 12*S*, 13*R*, 14*E*)-9-Acetoxy-13-(phenylthio)-11-(triethylsilyloxy) prosta-5,14-dienoate (23). To a stirred solution of the alcohol **21** (19 mg, 0.037 mmol) in CH_2Cl_2 (1 mL) at 0°C were added imidazole (6.3 mg, 0.092 mmol), 4-DMAP (0.9 mg, 0.007 mmol), and TESCl (12 mL, 0.074 mmol). After an additional 30 min, the reaction mixture was partitioned between CH_2Cl_2 and, sequentially, saturated aqueous NH_4Cl and brine. The organic extract was dried (Na_2SO_4) and concentrated. The residue was chromatographed to afford the TES-ether **23** (23.2 mg, quant.) as a colorless oil; TLC R_f (petroleum ether / MTBE = 7 / 3) = 0.31; ^{1}H NMR δ 7.33 - 7.36 (m, 2H), 7.18 - 7.28 (m, 3H), 5.28 - 5.41 (m, 3H), 5.04 (dt, 1H, J = 6.8 and 15.1 Hz), 4.95 (m, 1H), 4.35 (ddd, 1H, J = 3.7, 5.8, and 7.8 Hz), 4.12 (q, 2H, J = 7.1 Hz), 3.60 (t, 1H, J = 8.8 Hz), 2.57 (quint, 1H, J = 7.5 Hz), 2.44 (q, 1H, J = 7.5 Hz), 2.24 - 2.32 (m, 2H), 2.27 (t, 2H, J = 7.5 Hz), 2.03 (s, 3H), 1.95 - 2.05 (m, 2H), 1.87 - 1.92 (m, 1H), 1.82 (q, 2H, J = 6.8 Hz), 1.60 - 1.70 (m, 3H), 1.25 (t, 3H, J = 7.1 Hz), 1.0 - 1.22 (m, 6H), 0.98 (t, 9H, J = 7.8 Hz), 0.83 (t, 3H, J = 7.1 Hz), 0.64 (q, 6H, J = 7.8 Hz); ^{13}C NMR δ up: 173.5, 170.9, 135.3, 60.2, 41.5, 33.7, 32.0, 31.2, 28.9, 26.8, 25.1, 24.8, 22.5, 5.0; down: 133.6, 132.2, 130.1, 129.3, 128.4, 127.0, 78.0, 74.2, 53.3, 52.4, 46.7, 21.4, 14.2, 14.0, 6.9; IR (film) 2955, 1738, 1459, 1374, 1245, 1096, 1024, 746 cm^{-1}; FAB MS *m/z* (rel intensity) 653 (M^+ + Na, 81), 461 (57), 330 (30), 329 (100), 241 (34), 219 (35), 185 (30), 133 (48), 117 (38), 115 (83); FAB HRMS calcd for $C_{36}H_{58}O_5SiSNa$ 653.3672, found 653.3647; $[\alpha]^{20}_D$ +8.4 (*c* 1.2, $CHCl_3$).

Methyl (5*Z*, 8*R*, 9*R*, 11*S*, 12*S*, 13*R*, 14*E*)-9-Hydroxy-13-(phenylthio)-11-(triethylsilyloxy) prosta-5,14-dienoate (24). To a stirred solution of the acetate **23** (22 mg, 0.035 mmol) in EtOH (2 mL) at rt was added K_2CO_3 (24 mg, 0.17 mmol). After an additional 1.5 h at 65°C, the reaction mixture was partitioned between EtOAc and, sequentially, saturated aqueous NH_4Cl and brine. The organic extract was dried (Na_2SO_4) and concentrated. The residue was dissolved in MeOH (2 mL). To this solution at rt was added K_2CO_3 (24 mg, 0.17 mmol). After an additional 1.5 h at 65°C, the reaction mixture was partitioned between EtOAc and, sequentially, saturated aqueous NH_4Cl and brine. The organic extract was dried (Na_2SO_4) and concentrated. The residue was chromatographed to afford the methyl ester **24** (13 mg, 66%) as a colorless oil; TLC Rf (petroleum ether / MTBE = 75 / 25) = 0.4; ^1H NMR δ 7.34 - 7.37 (m, 2H), 7.19 - 7.28 (m, 3H), 5.33 - 5.42 (m, 3H), 5.04 (dt, 1H, *J* = 6.8 and 15.1 Hz), 4.40 (quint, 1H, *J* = 3.8 Hz), 4.07 - 4.10 (m, 1H), 3.66 (s, 3H), 3.57 (t, 1H, *J* = 8.6 Hz), 2.50 (dt, 1H, *J* = 3.8 and 7.8 Hz), 2.38 (dt, 1H, *J* = 6.6 and 14.4 Hz), 2.29 (t, 2H, *J* = 7.5 Hz), 2.18 - 2.29 (m, 2H), 1.94 - 2.06 (m, 4H), 1.84 (q, 1H, *J* = 7.2 Hz), 1.61 - 1.71 (m, 4H), 1.01 - 1.25 (m, 6H), 0.98 (t, 9H, *J* = 7.8 Hz), 0.84 (t, 3H, *J* = 7.2 Hz), 0.65 (q, 6H, *J* = 7.8 Hz); ^{13}C NMR δ up: 174.0, 135.2, 43.6, 33.4, 32.1, 31.2, 28.9, 26.8, 25.8, 24.7, 22.5, 5.0; down: 133.5, 132.0, 129.8, 129.7, 129.3, 128.5, 127.1, 76.5, 74.8, 53.9, 52.8, 51.5, 50.2, 14.0, 7.0; IR (film) 3447, 2954, 1740, 1458, 1240, 1091, 1006, 745 cm^{-1}; FAB MS *m/z* (rel intensity) 597 (M^+ + Na, 2), 465 (23), 447 (49), 435 (21), 316 (28), 315 (95), 307 (28), 301 (22), 289 (47), 257 (43), 219 (100); FAB HRMS calcd for $C_{33}H_{54}O_4SiSNa$ 597.3410, found 597.3397; $[\alpha]^{20}_D$ +12.9 (*c* 0.55, $CHCl_3$).

Methyl (5*Z*, 8*R*, 11*S*, 12*S*, 13*R*, 14*E*) -9-Oxo-13-(phenylthio)-11-(triethylsilyloxy)prosta-5,14-dienoate (25). To a stirred solution of the alcohol **24** (8 mg, 0.014 mmol) in CH_2Cl_2 (1 mL) at rt was added Dess-Martin periodinane (9 mg, 0.021 mmol). After an additional 1.5 h, the reaction was cooled in an ice bath. The resulting precipitate was filtered and washed with Et_2O. Evaporation of the filtrate gave a residue that was chromatographed to afford the ketone **25** (7.5 mg, 94%) as a colorless oil; TLC Rf (petroleum ether / MTBE = 75 / 25) = 0.71; ^1H NMR δ 7.19 - 7.30 (m, 5H), 5.57 (dd, 1H, *J* = 8.0 and 15.1 Hz), 5.35 - 5.46 (m, 3H), 4.54 (d, 1H, *J* = 6.4 Hz), 3.66 (s, 3H), 3.51 (dd, 1H, *J* = 4.1 and 8.0 Hz), 2.77 - 2.82 (m, 1H), 2.75 (dd, 1H, *J* = 6.4 and 19.6 Hz), 2.59 - 2.67 (m, 2H), 2.19 - 2.31 (m, 4H), 1.97 (q, 2H, *J* = 6.8 Hz), 1.90 (q, 2H, *J* = 7.6 Hz), 1.57 - 1.63 (m, 2H), 1.11 - 1.31 (m, 6H), 0.95 (t, 9H, *J* = 7.9 Hz), 0.86 (t, 3H, *J* = 7.1 Hz), 0.61 (q, 6H, *J* = 7.9 Hz); ^{13}C NMR δ up: 217.2, 174.0, 134.9, 47.5, 33.5, 32.1, 31.3, 28.8, 26.8, 24.7, 22.8, 22.5, 4.7; down: 133.5, 132.1, 130.2, 129.1, 128.7, 128.2,127.2, 68.6, 53.0, 51.5, 51.2, 50.2, 14.0, 6.8; IR (film) 2955, 1743, 1438, 1241, 1174, 1066, 1010, 746 cm^{-1}; FAB MS *m/z* (rel intensity) 595 (M^+ + Na, 43), 573 (64), 465 (33), 464 (37), 463 (100), 461 (51), 391 (37), 369 (85), 331 (57), 299 (85); FAB HRMS calcd for $C_{33}H_{52}O_4SiSNa$ 595.3253, found 595.3232; $[\alpha]^{20}_D$ -61.4 (*c* 0.36, $CHCl_3$).

Methyl (5*Z*, 8*R*, 11*S*, 12*S*, 13*E*, 15*R*)-15-Hydroxy-9-oxo-11-(triethylsilyloxy)prosta-5,13-dienoate (26). To a stirred solution of the thioether **25** (5.2 mg, 0.009 mmol) in CH_2Cl_2 (0.7 mL) at -78°C was added a solution of mCPBA (2.4 mg, 0.014 mmol) in CH_2Cl_2 (0.3 mL). The mixture was stirred for 1 h, after which a solution of trimethyl phosphite (11 mL, 0.09 mmol) in MeOH (0.5 mL) was added. The mixture was stirred at -78°C for 5 min and then warmed to rt. The reaction mixture was partitioned between EtOAc and, sequentially, saturated aqueous $NaHCO_3$ and brine. The organic extract was dried (Na_2SO_4) and concentrated. The residue was chromatographed to afford the alcohol **26** (3.8 mg, 87%) as a colorless oil; TLC Rf

(petroleum ether / MTBE = 6 / 4) = 0.55; ^{1}H NMR δ 5.65 (dd, 1H, *J* = 6.2 and 15.1 Hz), 5.34 - 5.42 (m, 2H), 5.26 (dd, 1H, *J* = 10.1 and 15.3 Hz), 4.24 (d, 1H, *J* = 5.4 Hz), 4.06 (q, 1H, *J* = 6.2 Hz), 3.66 (s, 3H), 2.95 (t, 1H, *J* = 8.8 Hz), 2.71 - 2.77 (m, 1H), 2.47 (dd, 2H, *J* = 5.4 and 18.8 Hz), 2.30 (t, 2H, *J* = 7.4 Hz), 2.02 - 2.08 (m, 2H), 1.85 - 1.93 (m, 1H), 1.70 - 1.82 (br s, 1H), 1.67 (q, 2H, *J* = 7.4 Hz), 1.44 - 1.63 (m, 3H), 1.25 - 1.39 (m, 6H), 0.95 (t, 9H, *J* = 7.9 Hz), 0.88 (t, 3H, *J* = 6.8 Hz), 0.60 (q, 6H, *J* = 7.9 Hz); ^{13}C NMR δ up: 217.7, 174.2, 45.6, 37.3, 33.4, 31.7, 26.7, 25.0, 24.7, 23.0, 22.6, 4.7; down: 137.3, 129.9, 127.8, 126.7, 72.5, 72.3, 51.9, 51.6, 50.3, 14.0, 6.8; IR (film) 3468, 2930, 1743, 1459, 1242, 1162, 1068, 1014, 746 cm^{-1}; FAB MS *m/z* (rel intensity) 503 (M^+ + Na, 100), 463 (18); FAB HRMS calcd for $C_{27}H_{48}O_5SiNa$ 503.3169, found 503.3163; $[\alpha]^{20}_D$ -55.8 (c 0.19, $CHCl_3$).

***ent*-15-E2t-Isoprostane Methyl Ester (27).** To a stirred solution of the silyl ether **26** (3.3 mg, 0.0069 mmol) in CH_3CN (1 mL) at 0°C was added pyridine (30 mL) followed by 52% aqueous HF solution (50 mL). After an additional 2 h, the reaction mixture was poured into saturated aqueous $NaHCO_3$ and then extracted with CH_2Cl_2. The organic extract was washed with brine and dried (Na_2SO_4), and concentrated. The residue was chromatographed to afford *ent*-15-E2t-isoprostane methyl ester (**27**) (2.13 mg, 85%) as a colorless oil; TLC Rf (petroleum ether / MTBE = 2 / 8) = 0.23; ^{1}H NMR δ 5.68 (dd, 1H, *J* = 6.1 and 15.3 Hz), 5.29 - 5.40 (m, 3H), 4.36 - 4.39 (m, 1H), 4.06 - 4.11 (m, 1H), 3.67 (s, 3H), 2.97 - 3.01 (m, 1H), 2.71 - 2.77 (m, 1H), 2.56 (dd, *J* = 5.8 and 19.2 Hz), 2.41 - 2.48 (m, 1H), 2.30 - 2.36 (m, 1H), 2.31 (t, 2H, *J* = 7.4 Hz), 1.93 - 2.08 (m, 3H), 1.40 - 1.70 (m, 6H), 1.19 - 1.39 (m, 6H), 0.88 (t, 3H, *J* = 6.8 Hz); ^{13}C NMR δ up: 216.6, 174.2, 44.7, 37.3, 33.4, 31.7, 26.7, 25.1, 24.6, 23.2, 22.6; down: 137.7, 130.1, 127.5, 126.1, 72.2, 72.2, 51.6, 51.4, 50.6, 14.0; IR (film) 3402, 2923, 2852, 1738, 1462, 1260, 1163, 1090, 1031, 799 cm^{-1}; $[\alpha]^{20}_D$ -62.0 (*c* 0.075, MeOH) (lit. [8] $[\alpha]_D$ +40.95 (c 0.075, MeOH).

Ethyl (5*Z*,8*R*,9*R*,11*S*,12*S*,13*E*,15*R*)-9-Acetoxy-11,15-dihydroxyprosta-5,13-dienoate (28). To a stirred solution of the thioether **21** (82 mg, 0.16 mmol) in CH_2Cl_2 (2 mL) at -78°C was added a solution of mCPBA (41 mg, 0.24 mmol) in CH_2Cl_2 (1 mL). The mixture was stirred for 1.5 h, after which a solution of trimethyl phosphite (187 mL, 1.59 mmol) in EtOH (1 mL) was added. The mixture was stirred at -78°C for 5 min and then warmed to rt. The reaction mixture was partitioned between EtOAc and, sequentially, saturated aqueous $NaHCO_3$ and brine. The organic extract was dried (Na_2SO_4) and concentrated. The residue was chromatographed to afford the alcohol **28** (67.2 mg, 99.7%) as a colorless oil; TLC Rf (EtOAc / petroleum ether = 6 / 4) = 0.24; ^{1}H NMR δ 5.60 (dd, 1H, *J* = 6.8 and 15.3 Hz), 5.49 (dd, 1H, *J* = 9.2 and 15.3 Hz), 5.34 - 5.42 (m, 2H), 4.84 (dt, 1H, *J* = 4.3 and 7.6 Hz), 4.13 (q, 2H, *J* = 7.1 Hz), 4.03 - 4.15 (m, 2H), 2.67 - 2.73 (m, 1H), 2.62 (quint, 1H, *J* = 7.6 Hz), 2.40 - 2.80 (br, 2H), 2.29 (t, 2H, *J* = 7.4 Hz), 2.27 - 2.34 (m, 1H), 2.04 (s, 3H), 1.94 - 2.12 (m, 4H), 1.41 - 1.71 (m, 5H), 1.24 - 1.39 (m, 6H), 1.26 (t, 3H, *J* = 7.1 Hz), 0.88 (t, 3H, *J* = 6.8 Hz); ^{13}C NMR δ up: 173.7, 170.8, 60.3, 39.8, 37.1, 33.6, 31.7, 26.7, 26.2, 25.1, 24.6, 22.6; down: 136.6, 129.9, 128.5, 128.4, 77.8, 75.4, 72.8, 53.0, 47.3, 21.3, 14.2, 14.0; IR (film) 3408, 2932, 1733, 1445, 1374, 1246, 1033, 973 cm^{-1}; FAB MS *m/z* (rel intesity) 447 (M^+ + Na, 1), 425 (36), 407 (48), 347 (30), 330 (28), 329 (100); FAB HRMS calcd for $C_{24}H_{40}O_6Na$ 447.2723, found 447.2713; $[\alpha]^{20}_D$ +15.5 (*c* 0.32, $CHCl_3$).

Ethyl (5*Z*,8*R*,9*R*,11*S*,12*S*,13*E*)-9-Acetoxy-11-hydroxy-15-oxoprosta-5,13-dienoate (29). To a stirred solution of the diol **28** (58 mg, 0.14 mmol) in 1,4-dioxane-CH_2Cl_2 (1 : 1, 2 mL) at rt

was added DDQ (78 mg, 0.34 mmol). After an additonal 17 h at 40°C, the solvent was evaporated to leave a residue. Treatment of the residue with CH_2Cl_2 left undissolved material. The resulting precipitate was filtered and washed with CH_2Cl_2. Evaporation of the filtrate gave a residue that was chromatographed to afford the enone **29** (38.8 mg, 67%) as a colorless oil; TLC Rf (petroleum ether / EtOAc = 6 / 4) = 0.29; ^{1}H NMR δ 6.68 (dd, 1H, *J* = 9.3 and 15.7 Hz), 6.23 (dd, 1H, *J* = 0.9 and 15.7 Hz), 5.31 - 5.44 (m, 2H), 4.89 (quint, 1H, *J* = 4.0 Hz), 4.18 - 4.22 (m, 1H), 4.12 (q, 2H, *J* = 7.1 Hz), 2.87 - 2.92 (m, 1H), 2.68 (quint, 1H, *J* = 7.6 Hz), 2.53 (t, 2H, *J* = 7.4 Hz), 2.41 - 2.48 (m, 1H), 2.22 - 2.30 (m, 1H), 2.28 (t, 2H, *J* = 7.4 Hz), 2.06 (s, 3H), 1.99 - 2.06 (m, 4H), 1.57 - 1.72 (m, 5H), 1.24 - 1.36 (m, 4H), 1.25 (t, 3H, *J* = 7.1 Hz), 0.90 (t, 3H, *J* = 7.0 Hz); ^{13}C NMR δ up: 200.1, 173.5, 170.7, 60.2, 40.6, 40.2, 33.6, 31.4, 26.7, 26.3, 24.6, 23.7, 22.4; down: 143.3, 132.1, 130.4, 127.7, 77.7, 74.9, 52.9, 47.7, 21.2, 14.2, 13.9; IR (film) 3452, 2933, 1733, 1670, 1626, 1457, 1374, 1245, 1182, 1048, 981, 728 cm^{-1}; EI MS *m/z* (rel intesity) 422 (M^+, 29), 344 (100), 273 (80), 247 (75), 245 (73), 230 (51), 189 (54), 117 (58); HRMS calcd for $C_{24}H_{38}O_6$ 422.2668, found 422.2659; $[\alpha]^{20}_D$ +10.6 (*c* 0.78, $CHCl_3$).

Reduction of the enone 29. To a stirred solution of the enone **29** (36 mg, 0.085 mmol) in MeOH (1 mL) at 0°C was added $NaBH_4$ (9.7 mg, 0.256 mmol). After an additional 1 h, the reaction mixture was partitioned between EtOAc and, sequentially, saturated aqueous NH_4Cl and brine. The organic extract was dried (Na_2SO_4) and concentrated. The residue was chromatographed to afford the 15-*S* alcohol **30** (16.5 mg, 46%) as a colorless oil; TLC Rf (petroleum ether / EtOAc = 1 / 1) = 0.32; ^{1}H NMR δ 5.64 (dd, 1H, *J* = 6.2 and 15.4 Hz), 5.48 (dd, 1H, *J* = 9.2 and 15.4 Hz), 5.35 - 5.43 (m, 2H), 4.86 (ddd, 1H, *J* = 3.8, 5.3, and 7.6 Hz), 4.13 (q, 2H, *J* = 7.1 Hz), 4.06 - 4.11 (m, 2H), 2.70 - 2.5 (m, 1H), 2.63 (quint, 1H, *J* = 7.6 Hz), 2.32 - 2.39 (m, 1H), 2.29 (t, 2H, *J* = 7.2 Hz), 2.05 (s, 3H), 1.80 - 2.14 (m, 6H), 1.44 - 1.70 (m, 5H), 1.24 - 1.4 (m, 6H), 1.26 (t, 3H, *J* = 7.1 Hz), 0.89 (t, 3H, *J* = 6.8 Hz); ^{13}C NMR δ up: 173.8, 170.9, 60.4, 40.1, 37.2, 33.6, 31.7, 26.7, 26.2, 25.2, 24.7, 22.6; down: 136.9, 129.8, 128.4, 127.6, 77.9, 75.7, 72.6, 53.0, 47.1, 21.3, 14.2, 14.0; IR (film) 3425, 2931, 1734, 1456, 1375, 1246, 1050, 973 cm^{-1}; FAB MS *m/z* (rel intesity) 447 (M^+ + Na, 2), 425 (16), 409 (62), 347 (32), 330 (29), 329 (100); FAB HRMS calcd for $C_{24}H_{40}O_6Na$ 447.2723, found 447.2726; $[\alpha]^{20}_D$ +10.1 (*c* 0.32, $CHCl_3$). This was followed by the 15-*R* alcohol **28** (13.3 mg, 37%) as a colorless oil; TLC Rf (petroleum ether / EtOAc = 1 / 1) = 0.27.

***ent*-15-F_{2t}-Isoprostane (6).** To a stirred solution of the acetate **28** (12 mg, 0.028 mmol) in THF-H_2O (1 : 1, 1.2 mL) at rt was added $LiOH•H_2O$ (11.9 mg, 0.28 mmol). After an additional 3 h, the reaction mixture at 0°C was acidified to pH 4 by adding 1% HCl (1.2 mL). After the addition of solid NaCl (1 g), the mixture was extracted with $CHCl_3$. The combined organic extract was dried (Na_2SO_4) and concentrated. The residue was chromatographed to afford *ent*-15-F_{2t}-isoprostane (**6**) (9 mg, 90%) as a colorless oil; TLC Rf (EtOAc / MeOH / AcOH = 80 / 20 / 0.1) = 0.32; ^{1}H NMR (CD_3OD) δ 5.35 - 5.54 (m, 4H), 3.4 - 4.90 (m, 2H), 3.86 (dt, 1H, *J* = 5.6 and 7.5 Hz), 2.65 - 2.70 (m, 1H), 2.48 (quint, 1H, *J* = 7.4 Hz), 2.29 (t, 2H, *J* = 7.4 Hz), 1.98 - 2.19 (m, 5H), 1.65 (quint, 1H, *J* = 7.4 Hz), 1.24 - 1.58 (m, 9H), 0.90 (t, 3H, *J* = 6.8 Hz); ^{13}C NMR (CD_3OD) δ up: 43.5, 38.4, 33.0, 27.8, 27.4, 26.3, 26.2, 23.7; down: 136.9, 130.5, 130.4, 76.3, 76.2, 73.7, 53.8, 51.4, 14.4; IR (film) 3342, 2924, 1713, 1456, 1260, 1074, 972 cm^{-1}; FAB MS *m/z* (rel intesity) 377 (M^+ + Na, 100), 371 (67), 343 (27), 333 (33), 305

(21); FAB HRMS calcd for $C_{20}H_{34}O_5Na$ 377.2304, found 377.2300; $[\alpha]^{20}_D$ -7.7 (*c* 0.46, MeOH).

***ent*-15-*epi*-F_{2t}-Isoprostane (7).** This reaction was performed with 15 mg (0.035 mmol) of the acetate **30**, LiOH•H_2O (14.8 mg, 0.35 mmol), and THF-H_2O (1 : 1, 1.4 mL) in the same manner as described for the preparation of *ent*-15-F_{2t}-isoprostane (**6**) to give *ent*-15-*epi*-F_{2t}-isoprostane (**7**) (12.3 mg, 98%) as a colorless oil; TLC R_f (EtOAc / MeOH / AcOH = 80 / 20 / 0.1) = 0.27; ^{1}H NMR (CD_3OD) δ 5.35 - 5.57 (m, 4H), 4.00 (q, 1H, *J* = 6.1 Hz), 3.96 (dt, 1H, *J* = 4.8 and 7.2 Hz), 3.87 (dt, 1H, *J* = 5.1 and 7.5 Hz), 2.65 - 2.72 (m, 1H), 2.48 (quint, 1H, *J* = 7.2 Hz), 2.31 (br, 2H), 2.03 - 2.16 (m, 5H), 1.65 (quint, 1H, *J* = 7.2 Hz), 1.24 - 1.58 (m, 9H), 0.90 (t, 3H, *J* = 6.8 Hz); ^{13}C NMR (CD_3OD) δ up: 43.6, 38.5, 33.0, 27.8, 27.3, 26.3, 26.2, 23.7; down: 136.7, 130.52, 130.45, 129.9, 76.23, 76.15, 73.5, 53.5, 51.4, 14.4; IR (film) 3322, 2926, 1705, 1239, 1063, 971 cm^{-1}; FAB MS *m/z* (rel intesity) 377 (M^+ + Na, 100), 359 (6), 319 (4); FAB HRMS calcd for $C_{20}H_{34}O_5Na$ 377.2304, found 377.2300; $[\alpha]^{20}_D$ -4.70 (*c* 0.58, MeOH).

Ethyl (5*Z*,8*S*,9*S*,11*R*,12*R*,13*E*,15*S*)-11-Acetoxy-9,15-dihydroxyprosta-5,13-dienoate (31). To a stirred solution of the thioether **22** (86 mg, 0.17 mmol) in CH_2Cl_2 (2 mL) at -78°C was added a solution of mCPBA (57 mg, 0.33 mmol) in CH_2Cl_2 (1 mL). The mixture was stirred for 1.5 h, after which a solution of trimethyl phosphite (197 mL, 1.67 mmol) in EtOH (1 mL) was added. The mixture was stirred at -78°C for 5 min and then warmed to rt. The reaction mixture was partitioned between EtOAc and, sequentially, saturated aqueous $NaHCO_3$ and brine. The organic extract was dried (Na_2SO_4) and concentrated. The residue was chromatographed to afford the alcohol **31** (70.5 mg, 99.8%) as a colorless oil; TLC R_f (EtOAc / petroleum ether = 6 / 4) = 0.32; ^{1}H NMR δ 5.58 (dd, 1H, *J* = 6.2 and 15.4 Hz), 5.39 - 5.50 (m, 3H), 4.94 (quint, 1H, *J* = 3.8 Hz), 4.13 (q, 2H, *J* = 7.1 Hz), 4.08 (q, 1H, *J* = 6.2 Hz), 4.01 (dt, 1H, *J* = 4.8 and 7.6 Hz), 2.88 - 2.93 (m, 1H), 2.61 (quint, 1H, *J* = 7.6 Hz), 2.31 (t, 2H, *J* = 7.2 Hz), 2.05 (s, 3H), 1.99 - 2.11 (m, 7H), 1.62 - 1.72 (m, 3H), 1.44 - 1.55 (m, 2H), 1.24 - 1.43 (m, 6H), 1.26 (t, 3H, *J* = 7.1 Hz), 0.88 (t, 3H, *J* = 6.8 Hz); ^{13}C NMR δ up: 173.8, 170.8, 60.4, 40.1, 37.1, 33.6, 31.7, 26.7, 26.4, 25.1, 24.7, 22.6; down: 136.5, 130.1, 128.8, 127.2, 77.8, 75.8, 72.5, 50.5, 49.8, 21.3, 14.2, 14.0; IR (film) 3428, 2931, 1733, 1456, 1375, 1247, 1032, 971 cm^{-1}, FAB MS *m/z* (rel intensity) 447 (M^+ + Na, 2), 425 (18), 407 (42), 347 (35), 330 (28), 329 (100), 257 (41); FAB HRMS calcd for $C_{24}H_{40}O_6Na$ 447.2723, found 447.2709; $[\alpha]^{20}_D$ +7.1 (*c* 0.23, $CHCl_3$).

Ethyl (5*Z*, 8*S*, 9*S*, 11*R*, 12*R*, 13*E*)-11-Acetoxy-9-hydroxy-15-oxoprosta-5,13-dienoate (32). This reaction was performed with 63 mg (0.15 mmol) of the diol **31**, DDQ (84 mg, 0.37 mmol), and 1,4-dioxane-CH_2Cl_2 (1 : 1, 2.4 mL) in the same manner as described for the preparation of the enone **29** to give the enone **32** (53.5 mg, 85%) as a colorless oil; TLC R_f (petroleum ether / EtOAc = 6 : 4) = 0.34; ^{1}H NMR δ 6.67 (dd, 1H, *J* = 9.1 and 15.8 Hz), 6.18 (dd, 1H, *J* = 1.1 and 15.8 Hz), 5.36 - 5.47 (m, 2H), 5.01 (dt, 1H, *J* = 4.3 and 8.1 Hz), 4.13 (q, 2H, *J* = 7.2 Hz), 4.04 - 4.08 (m, 1H), 3.08 - 3.13 (m, 1H), 2.66 (quint, 1H, *J* = 7.6 Hz), 2.53 (t, 2H, *J* = 7.4 Hz), 2.30 (t, 2H, *J* = 7.4 Hz), 2.18 - 2.25 (m, 2H), 2.05 (s, 3H), 1.95 - 2.10 (m, 4H), 1.57 - 1.73 (m, 5H), 1.24 - 1.36 (m, 4H), 1.26 (t, 3H, *J* = 7.2 Hz), 0.90 (t, 3H, *J* = 7.0 Hz); ^{13}C NMR δ up: 200.1, 173.6, 170.7, 60.3, 40.7, 40.1, 33.6, 31.4, 26.7, 26.4, 24.6, 23.7, 22.4; down: 142.8, 131.8, 130.6, 128.1, 76.7, 75.3, 51.0, 49.6, 21.1, 14.2, 13.9; IR (film) 3458, 2933, 1735, 1671, 1627, 1444, 1373, 1244, 1179, 1048, 980, 729 cm^{-1}; EI MS *m/z* (rel intesity) 377 (M^+, 19), 377 (25), 362

(55), 306 (40), 248 (100), 247 (74), 245 (27), 203 (25), 199 (25), 151 (26), 117 (29), 105 (26); HRMS calcd for $C_{24}H_{38}O_6$ 422.2668, found 422.2688; $[\alpha]\ ^{20}D$ -13.8 (*c* 0.84, $CHCl_3$).

Reduction of the enone 32. This reaction was performed with 51 mg (0.12 mmol) of the enone **32**, $NaBH_4$ (13.7 mg, 0.36 mmol), and MeOH (1.4 mL) in the same manner as described for the reduction of the enone **29** to give the 15-*R* alcohol **33** (19.9 mg, 39%) as a colorless oil; TLC Rf (petroleum ether / MTBE = 2 / 8) = 0.40; ^1H NMR δ 5.59 (dd, 1H, *J* = 6.5 and 15.6 Hz), 5.40 - 5.49 (m, 3H), 4.92 (quint, 1H, *J* = 3.8 Hz), 4.13 (q, 2H, *J* = 7.1 Hz), 4.07 (q, 1H, *J* = 6.5 Hz), 3.81 - 4.02 (m, 1H), 2.91 (m, 1H), 2.61 (quint, 1H, *J* = 7.6 Hz), 2.31 (t, 2H, *J* = 7.1 Hz), 2.05 (s, 3H), 2.04 - 2.13 (m, 5H), 1.86 - 1.98 (br, 2H), 1.43 (m, 5H), 1.23 - 1.40 (m, 6H), 1.26 (t, 3H, *J* = 7.1 Hz), 0.88 (t, 3H, *J* = 6.8 Hz); ^{13}C NMR δ up: 173.8, 170.7, 60.4, 40.1, 37.2, 33.5, 31.7, 26.7, 26.4, 25.1, 24.7, 22.6; down: 136.7, 130.2, 128.9, 127.2, 77.8, 75.8, 72.7, 50.5, 49.9, 21.3, 14.2, 14.0; IR (film) 3434, 2931, 1735, 1456, 1374, 1246, 1027, 973 cm^{-1}; FAB MS *m/z* (rel intesity) 447 (M^+ + Na, 2), 408 (24), 407 (89), 347 (45), 330 (32), 329 (100), 301 (22), 257 (41); FAB HRMS calcd for $C_{24}H_{40}O_6Na$ 447.2723, found 447.2707; $[\alpha]^{20}D$ -19.0 (*c* 0.37, $CHCl_3$). This was followed by the 15-*R* alcohol **31** (19.3 mg, 38%) as a colorless oil; TLC Rf (petroleum ether / MTBE = 2 / 8) = 0.33.

15-F_{2t}-Isoprostane (4). This reaction was performed with 18 mg (0.042 mmol) of the acetate **31**, LiOH•H_2O (17.8 mg, 0.42 mmol), and THF-H_2O (1 : 1, 1.5 mL) in the same manner as described for the preparaton of *ent*-15-F_{2t}-isoprostane (**6**) to give 15-F_{2t}-isoprostane (**4**) (13 mg, 87%) as a colorless oil. This compound was identical with *ent*-15-F_{2t}-isoprostane (**6**) except for the specific optical rotation; $[\alpha]^{20}D$ +7.2 (*c* 0.58, MeOH).

15-*epi*-F_{2t}-Isoprostane (5). This reaction was performed with 18 mg (0.042 mmol) of the acetate **33**, LiOH•H2O (17.8 mg, 0.42 mmol), and THF-H_2O (1 : 1, 1.5 mL) in the same manner as described for the preparation of *ent*-15-F_{2t}-isoprostane (**6**) to give 15-*epi*-F_{2t}-isoprostane (**5**) (13.9 mg, 93%) as a colorless oil. This compound was identical with *ent*-15-*epi*-F_{2t}-isoprostane (**7**) except for the specific optical rotation; $[\alpha]^{20}D$ +4.85 (*c* 0.68, MeOH).

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