Synthesis of 2,3-Dinor-5,6-dihydro-15F_{2t}-isoprostane

Douglass F. Taber* and Kazuo Kanai

Department of Chemistry and Biochemistry, University of Delaware, Newark, Delaware 19716

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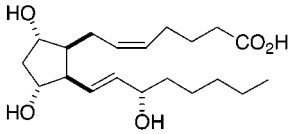
A concise synthesis of the 15-F_{2t}-isoprostane urinary metabolite **2**, a potential marker for systemic oxidative stress, is described. This synthesis confirmed the structure of this important product of human metabolism. The key transformation is the rhodium-mediated diastereoselective cyclization of diazo ketone **4**, followed by cyclopropane ring opening with thiophenol and Lewis acid. Material prepared by the synthesis outlined here will be used to develop an antibody-based assay for clinically quantifying systemic oxidative stress.

Introduction

It is increasingly apparent that hypercholesterolemia is linked to an increased risk of cardiovascular desease only under conditions of at least mild oxidative stress.[1] Recently, a series of bioactive prostaglandin-like compounds, the isoprostanes,[2] produced (as racemates) from membrane-bound arachidonic acid by free-radical oxidation, have been described.[3] These findings suggest that quantification of these isoprostanes (e.g., 15-F_{2t}-isoprostane **1**) may provide a practical approach to the assessment of systemic oxidative stress. Recently, Roberts and co-workers tentatively identified the major urinary metabolite of 15-F_{2t}-isoprostane (**1**) as 2,3-dinor-5,6-dihydro-15F_{2t}-isoprostane (**2**) by HPLC and mass spectrometric analysis.[4a] Before clinical assays based on **2** (preferred over measuring the unmetabolized isoprostanes[4b]) could be developed, it was important to first confirm the structure of this important product of human metabolism.[5−11]



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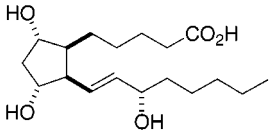

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Results and Discussion

Based on our prior work,[8] we proposed (Scheme 1) to prepare **2** by aldol condensation of the ketone **5** with the aldehyde **6**, followed by rhodium(II)-catalyzed cyclization of the silyloxy ketone **4**.

The diketone **8** (Scheme 2), prepared from benzoylacetone (**7**) and 5-bromovalerate, was subjected to the diazo-transfer reaction[12] with *p*-nitrobenzenesulfonyl azide (*p*-NBSA) and DBU to give the diazo ketone **5**. Aldol condensation of the diazo ketone **5** with (*E*,*E*)-decadienal **6** was successfully achieved by using KHMDS as base in the presence of triethylchlorosilane (TESCl), to produce the TES-protected aldol **9** together with a small amount of the free aldol **10** (hydrolysis on workup) in 70% combined yield.[6d]

We had shown[8] that the triethylsilyl protecting group was not stable to the conditions for cyclopropane opening, so this was changed to *tert*-butyldiphenylsilyl (TBDPS). The resulting diazo ketone **4** was then cyclized with catalytic rhodium(II) octanoate dimer to provide the bicyclic ketones **11** and **3** in 23% and 68% yields, respectively. The structures of the bicyclic ketones **3** and **11** were assigned by comparing their ^{1}H and ^{13}C NMR spectra to those for the analogous bicyclic ketones that are intermediates in the synthesis of the 15-F_{2t}-isopros-

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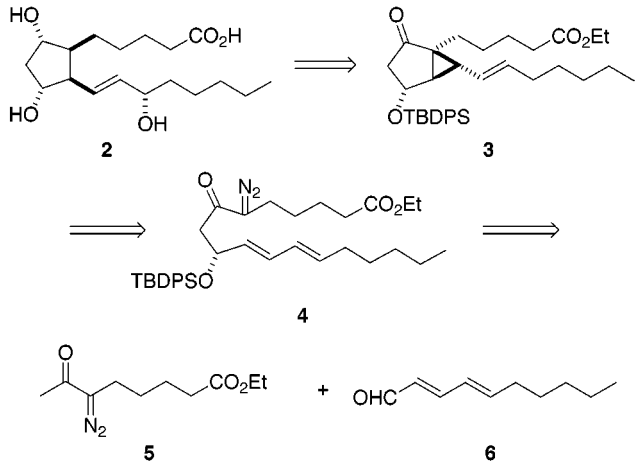
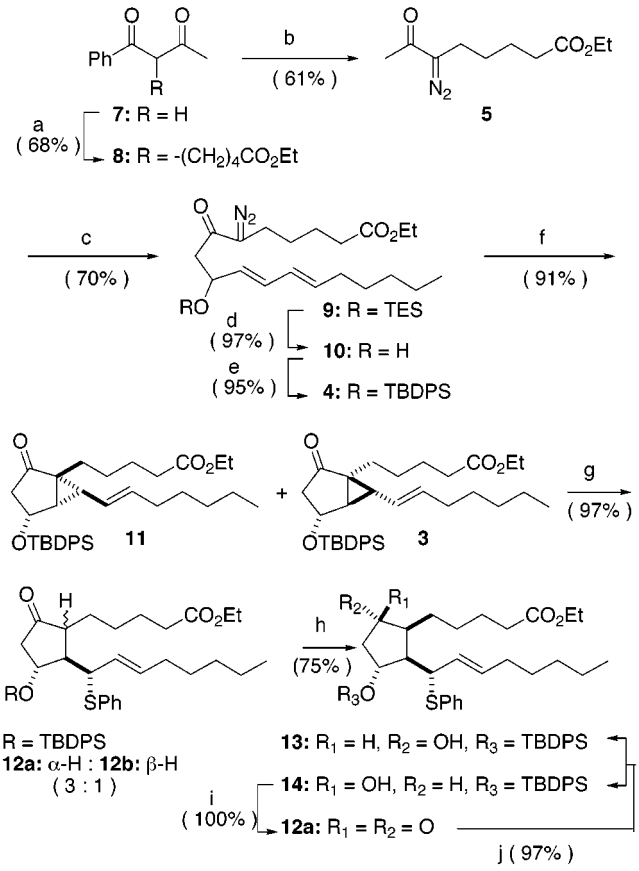
Scheme 1



Scheme 2[a]

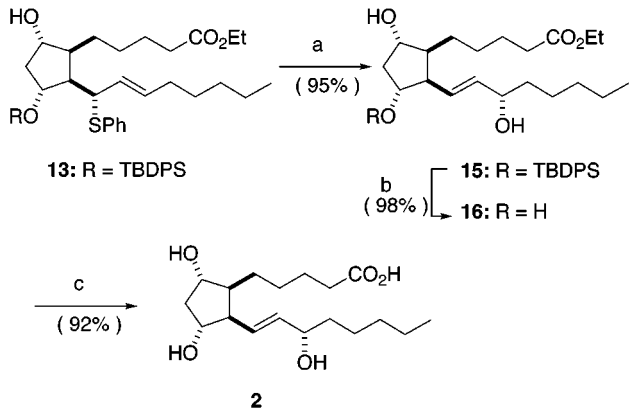


[a] Reagents and conditions: (a) 5-bromovalerate, K_2CO_3, *n*-Bu_4NBr, toluene, 100 °C then 60 °C; (b) *p*-NBSA, DBU, CH_2Cl_2, 0 °C; (c) KHMDS, (*E*,*E*)-decadienal **6**, TESCl, toluene, −78 °C (**9**:**10** = 3.5:1); (d) TBAF, NH_4Cl, THF, 0 °C; (e) TBDPS, imidazole, 4-DMAP, CH_2Cl_2; (f) $Rh_2(oct)_4$, CH_2Cl_2 (**11**:**3** = 1:3); (g) PhSH, $BF_3{\cdot}OEt_2$, CH_2Cl_2, −78 °C then −45 °C; (h) $NaBH_4$, MeOH, 0 °C (**13**: 45%; **14**: 30%); (i) Dess−Martin periodinane, CH_2Cl_2; (j) $NaBH_4$, MeOH, 0 °C (**13**: 58%; **14**: 39%).

tanes.[8] In particular, the oxygenated methine of **3** (^{13}C δ 69.3, ^1H δ 4.45, d, J = 4.9 Hz, 1H) is almost exactly congruent with the analogous 15-F_{2t}-isoprostane precursor (^{13}C δ 69.6, ^1H δ 4.41, d, 1H), while the oxygenated methine of **11** (^{13}C δ 67.9, ^1H δ 4.59, dt, J = 5.2, 7.9 Hz, 1H) is quite different.

Cyclopropane ring opening with thiophenol and $BF_3{\cdot}OEt_2$ in CH_2Cl_2 gave the ketones **12a** and **12b** as an

Scheme 3[a]



[a] Reagents and conditions: (a) *m*-CPBA, CH_2Cl_2, −78 °C; $(MeO)_3P$, EtOH, −78 °C ~ rt; (b) TBAF, THF; (c) $LiOH{\cdot}H_2O$; 1% aqueous HCl.

inseparable mixture (ca. 3:1) in 97% yield. These were distinguishable by comparing their ^{13}C NMR spectra with the spectra for the analogous ketones prepared in the synthesis of the 15-F_{2t}-isoprostanes.[8] Thus, ketone **12a** had methines at δ 52.4, 51.0, and 50.2, comparable to δ 52.4, 51.4, and 50.3 for the previous ketone with cis side chains, while ketone **12b** had methines at δ 53.3, 53.0, and 50.0, comparable to δ 53.1, 53.0, and 50.0 for the previous ketone with trans side chains. We assumed that the opening with thiophenol proceeded with inversion at the reacting center, as we have previously observed.[8]

Reduction of the ketone mixture with sodium borohydride produced alcohols **13** and **14** in 45% and 30% yields, respectively, accompanied by the alcohol from the reduction of **12b** (25%). Again, the relative configurations of **13** (^1H NMR δ 4.39, quint, J = 3.3 Hz, 1H; 3.98, m, 1H) and **14** (^1H NMR δ 4.64, dt, J = 3.6 and 6.1 Hz, 1H; 4.25, m, 1H) were assigned by comparison to the chemical shifts of the H's at C-9 and C-11 in the corresponding diastereomers of the 15-isoprostane intermediates (^1H NMR δ 4.38, m, 1H; 3.99, m, 1H) and (^1H NMR δ 4.64, m, 1H; 4.24, m, 1H).[8] The undesired β-alcohol **14** was oxidized by Dess−Martin periodinane[13] and then again reduced with $NaBH_4$ to give **13** and **14** in 58% and 39% yields, respectively, over two steps.

With the relative configurations around the ring of **13** firmly established, we were prepared to embark on the synthesis of 2,3-dinor-5,6-dihydro-15F_{2t} -isoprostane (**2**) (Scheme 3). Thus, oxidation and Mislow rearrangement[14] of the alcohol **13** gave the allylic alcohol **15**, which on treatment with TBAF in THF gave the triol **16** in 93% overall yield. Finally, the ester **16** was hydrolyzed with LiOH in THF−H_2O (1:1, v/v) to furnish the putative 15-F_{2t}-isoprostane urinary metabolite **2** in 92% yield.

Synthetic **2** was found[15] to co-chromatograph with the endogenously derived material on silica gel TLC (98:2 ethyl acetate/methanol) with an R_f = 0.21 when derivatized as the pentafluorobenzyl ester. Epimeric materials are easily discerned with this solvent system; for instance, the pentafluorobenzyl ester of $PGF_{2\alpha}$ and of its

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(15) We thank Dr. Jason Morrow at Vanderbilt University for making these comparisons.

8-epi diasteromer separated by 0.10 R_f units. In addition, synthetic **2** perfectly co-chromatographed with the endogenous metabolite on gas chromatography (DB1701 fused silica capillary column, methane as carrier gas, temperature ramp 190−300 °C at 20 °C per minute, retention time of 6 min) after derivatization to the pentafluorobenzyl ester/tris trimethylsilyl ether derivative. Again, epimeric materials are easily discerned with this system; the derivatives of $PGF_{2\alpha}$ and of its 8-epi diasteromer separated by a full 10 s.

Synthetic **2** was also analyzed[15,16] by MS in both the EI and negative ion CI modes. The synthetic material had a molecular weight and fragmentation pattern identical to the endogenously derived metabolite when analyzed both as the PFB ester TMS ether derivative and as the methyl ester TMS ether derivative.

Conclusions

We have confirmed the structure of the 15-F_{2t}-isoprostane urinary metabolite **2**, and have also developed a practical synthetic route to this important product of human metabolism. This racemic synthetic material (the natural metabolite is racemic) will be used to develop an immunoassay for the metabolite, thus allowing its quantification for an integrated assessment of individual oxidative stress.

Experimental Procedures[17]

Diketone 8. A mixture of benzoylacetone (**7**) (18.62 g, 114.8 mmol), K_2CO_3 (52.9 g, 382.6 mmol), and *n*-BuN_4Br (308 mg, 1.0 mmol) in toluene (300 mL) was heated at 100 °C for 3 h. The mixture was cooled at 60 °C for the addition of ethyl 5-bromovalerate (20 g, 95.7 mmol). The mixture was then stirred at 60 °C for 48 h. The mixture was further cooled to room temperature and 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 diketone **8** (18.8 g, 68% yield) as a yellow oil; TLC R_f (petroleum ether/MTBE = 6/4) = 0.49; ^{1}H NMR δ 7.97−8.00 (2H, m), 7.58−7.63 (1H, m), 7.43−7.51 (2H, m), 4.44 (1H, t, J = 7.0 Hz), 4.10 (2H, q, J = 7.1 Hz), 2.29 (2H, t, J = 7.4 Hz), 2.13 (3H, s), 1.91−2.14 (2H, m), 1.23 (3H, t, J = 7.1 Hz); ^{13}C NMR δ up 204.1, 196.2, 173.3, 136.3, 60.2, 33.8, 28.5, 27.0, 24.6; down 133.7, 128.8, 128.6, 63.1, 27.8, 14.1; IR (film) 2938, 1731, 1681, 1596, 1448, 1359, 1182, 1096, 1031, 972, 773, 695 cm^{-1}; CI MS m/z (rel intesity) 291 (M^+ + 1, 100), 275 (33), 249 (39), 204 (11), 187 (26), 163 (15), 157 (11), 141 (12), 105 (41); CI HRMS calcd for $C_{17}H_{23}O_4$ 291.1596, found 291.1596.

Diazo Ketone 5. To a stirred solution of the diketone **8** (5 g, 17.24 mmol) in CH_2Cl_2 (250 mmol) at 0 °C was added DBU (4.13 mL, 27.59 mmol). After 5 min, a solution of *p*-nitrobenzenesulfonyl azide (5.9 g, 25.86 mmol) in CH_2Cl_2 (100 mL) was added dropwise over 30 min. After an additional 1 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 diazo ketone **5** (2.23 g, 61% yield) as a pale yellow oil: TLC R_f (petroleum ether/MTBE = 6/4) = 0.31; ^{1}H NMR δ 4.13 (2H, q, J = 7.1 Hz), 2.34 (4H, m), 2.24 (3H, s), 1.68 (2H, quint, J = 7.6 Hz), 1.52 (2H, quint, J = 7.6 Hz), 1.26 (3H, t, J = 7.1 Hz); ^{13}C NMR δ up 191.0, 173.2, 66.9, 60.3, 33.7, 26.5, 23.9, 22.0; down 25.3, 14.1; IR (film) 2938, 2867, 2070, 1732, 1644, 1446, 1372, 1327, 1195, 1098, 1030, 958 cm^{-1}; EI MS m/z (rel intensity) 184 ($M^+ - N_2$, 100), 142 (57), 138 (89), 110 (82); EI HRMS calcd for $C_{10}H_{16}O_3$ 184.1099, found 184.1087.

TES-Protected Aldol 9 and Free Aldol 10. To a stirred solution of the diazo ketone **5** (1.92 g, 9.06 mmol) in toluene (190 mL) at −78 °C was added dropwise a 0.33 M toluene solution of KHMDS (30 mL, 9.96 mmol) over 15 min. After 5 min, a solution of (*E*,*E*)-decadienal **6** (1.65 g, 10.87 mmol) and TESCl (1.82 mL, 10.87 mmol) in toluene (40 mL) was added. Afetr 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-ether **9** (2.35 g, 54% yield) as a pale yellow oil: TLC R_f (petroleum ether/MTBE = 85/15) = 0.55; ^{1}H NMR δ 6.14 (1H, dd, J = 10.5, 15.1 Hz), 5.98 (1H, dd, J = 10.5, 15.1 Hz), 5.68 (1H, dt, J = 6.9, 15.1 Hz), 5.53 (1H, dd, J = 6.9, 15.1 Hz), 4.61 (1H, m), 4.12 (2H, q, J = 7.1 Hz), 2.73 (1H, dd, J = 8.2, 13.5 Hz), 2.46 (1H, dd, J = 4.6, 13.5 Hz), 2.28−2.43 (2H, m), 2.31 (2H, t, J = 7.3 Hz), 2.06 (2H, q, J = 6.9 Hz), 1.67 (2H, quint, J = 7.5 Hz), 1.50 (2H, quint, J = 7.5 Hz), 1.38 (2H, quint, J = 7.1 Hz), 1.23−1.37 (4H, m), 1.25 (3H, t, J = 7.1 Hz), 0.91 (9H, t, J = 8.0 Hz), 0.88 (3H, t, J = 7.1 Hz), 0.56 (6H, q, J = 8.0 Hz); ^{13}C NMR δ up 191.8, 173.3, 68.4, 60.3, 46.9, 33.8, 32.6, 31.4, 28.8, 26.6, 24.1, 22.5, 22.4, 4.8; down 135.7, 132.5, 130.5, 129.1, 71.1, 14.2, 14.0, 6.7; IR (film) 2955, 2875, 2070, 1736, 1631, 1459, 1368, 1239, 1181, 1071, 990, 744 cm^{-1}; FAB MS m/z (rel intensity) 501 (M^+ + Na, 48), 473 (50), 267 (100); FAB HRMS calcd for $C_{26}H_{46}N_2O_4NaSi$ 501.3125, found 501.3115. This was followed by the free aldol **10** (508 mg, 16% yield) as a colorless oil: TLC R_f (petroleum ether/MTBE = 85/15) = 0.09; ^{1}H NMR δ 6.25 (1H, dd, J = 10.4, 15.3 Hz), 6.01 (1H, dd, J = 10.4, 15.3 Hz), 5.72 (1H, dt, J = 7.0, 15.3 Hz), 5.58 (1H, dd, J = 7.0, 15.3 Hz), 4.63 (1H, q, J = 6.0 Hz), 4.13 (2H, q, J = 7.1 Hz), 3.30 (1H, br s), 2.67 (2H, br s, J = 6.0 Hz), 2.37 (2H, t, J = 7.4 Hz), 2.33 (2H, t, J = 7.4 Hz), 2.07 (2H, q, J = 6.9 Hz), 1.67 (2H, quint, J = 7.4 Hz), 15.2 (2H, quint, J = 7.4 Hz), 13.8 (2H, quint, J = 7.4 Hz), 1.24−1.34 (4H, m), 1.26 (3H, t, J = 7.1 Hz), 0.88 (3H, t, J = 7.1 Hz); ^{13}C NMR δ up 193.1, 173.3, 68.0, 60.4, 44.2, 33.7, 32.6, 31.3, 28.8, 26.5, 23.9, 22.5, 22.1; down 136.2, 131.2, 130.9, 129.1, 69.0, 14.2, 14.0; IR (film) 3437, 1928, 2858, 2074, 1732, 1621, 1462, 1372, 1183, 1076, 991, 875, 729 cm^{-1}; EI MS m/z (rel intensity) 336 ($M^+ - N_2$, 29), 290 (37), 191 (100), 110 (29); EI HRMS calcd for $C_{20}H_{32}O_4$ 336.2301, found 336.2320.

TBDPS-Protected Aldol 4. To a stirred solution of the TES-ether **9** (140 mg, 0.30 mmol) in THF (3 mL) at 0 °C was added NH_4Cl (78 mg, 1.46 mmol) followed by a 1.0 M THF solution of *n*-Bu_4NF (0.32 mL, 0.32 mmol). After an additional 30 min, 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 free aldol **10** (104 mg, 97% yield) as a pale yellow oil. To a stirred solution of the free aldol **10** (1.85 g, 5.08 mmol) in CH_2Cl_2 (40 mL) at room temperature were added imidazole (450 mg, 6.61 mmol), 4-DMAP (124 mg, 1.02 mmol), and *tert*-butylchlorodiphenylsilane (1.59 mL, 6.10 mmol). After 15 h at room temperature, 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 *tert*-butyldiphenylsilyl ether **4** (2.9 g, 95% yield) as a pale yellow oil: TLC R_f (petroleum ether/MTBE = 85/15) = 0.36; ^{1}H NMR δ 7.61−7.64 (4H, m), 7.31−7.42 (6H, m), 5.65−5.80 (2H, m), 5.38−5.48 (2H, m), 4.67 (1H, q, J = 6.7 Hz), 4.11 (2H, q, J = 7.1 Hz), 2.77 (1H, dd, J = 7.4, 13.6 Hz), 2.50 (1H, dd, J = 5.7, 13.7 Hz), 2.20−2.35 (4H, m), 1.99 (2H, q, J = 7.1 Hz), 1.63 (2H, quint, J = 7.5 Hz), 1.41−1.49 (2H, m), 1.22−1.37 (6H, m), 1.24 (3H, t, J = 7.1 Hz), 1.03 (9H, s), 0.88 (3H, t, J = 7.1 Hz); ^{13}C NMR δ up 191.3, 173.2, 133.8, 133.7, 68.1, 60.2, 46.4, 33.8, 32.5, 31.3, 28.8, 26.5, 24.0, 22.4, 22.2, 19.2; down 135.90, 135.85, 135.7, 131.4, 129.6, 129.6, 129.4, 129.0, 127.4, 127.3, 72.2, 26.9, 14.2, 14.0; IR (film) 2930, 2857, 2068, 1738, 1634, 1463, 1372, 1185, 1112, 1062, 989, 822, 703, 612 cm^{-1}; FAB MS m/z (rel intensity) 625 (M^+ + Na, 69),

(16) For full experimental details for the derivitization and analysis, see: Morrow, J. D.; Roberts, L. J., II. *Methods Enzymol.* **1999**, *300*, 3.

(17) For a summary of general experimental procedures, see: Taber, D. F.; Meagley, R. P.; Doren, D. J. *J. Org. Chem.* **1996**, *61*, 5723.

598 (46), 597 (100), 529 (45), 517 (21); FAB HRMS calcd for $C_{36}H_{50}N_2O_4NaS$ 625.3438.

Bicyclic Ketones 3 and 11. To a stirred solution of the diazo ketone **4** (1.5 g, 2.50 mmol) in CH_2Cl_2 (20 mL) at room temperature was added a solution of $Rh_2(oct)_4$ (0.7 mg, 0.013 mmol) in CH_2Cl_2 (10 mL). After 30 min at room temperature, the reaction mixture was concentrated. The residue was chromatographed to afford the bicyclic ketone **3** (977 mg, 68% yield) as a colorless oil: TLC R_f (benzene/MTBE = 97/3) = 0.50; ^{1}H NMR δ 7.60−7.69 (4H, m), 7.35−7.47 (6H, m), 5.44 (1H, dt, J = 8.0, 15.3 Hz), 4.99 (1H, dd, J = 8.0, 15.3 Hz), 4.45 (1H, d, J = 4.9 Hz), 4.11 (2H, q, J = 7.1 Hz), 2.31 (2H, dt, J = 2.6, 7.4 Hz), 2.22 (1H, dd, J = 5.0, 18.6 Hz), 1.95−2.04 (5H, m), 1.65−1.73 (2H, m), 1.43−1.55 (3H, m), 1.19−1.35 (7H, m), 1.24 (3H, t, J = 7.1 Hz), 1.05 (9H, s), 0.88 (3H, t, J = 7.1 Hz); ^{13}C NMR δ up 212.7, 173.6, 133.7, 133.5, 60.1, 43.8, 42.7, 34.2, 32.5, 31.2, 28.9, 27.1, 14.8, 23.1, 22.4, 19.0; down 135.7, 135.6, 134.6, 129.8, 127.7, 124.4, 69.3, 40.2, 31.7, 26.8, 14.2, 14.0; IR (film) 2930, 2857, 1732, 1463, 1428, 1372, 1302, 1245, 1161, 1112. 1062, 968, 906, 823, 703, 612 cm^{-1}; EI MS m/z (rel intensity) 574 (M^+, 34), 517 (47), 292 (47), 246 (90), 213 (99), 199 (100), 120 (46); EI HRMS calcd for $C_{36}H_{50}O_4Si$ 574.3478, found 574.3503. This was followed by the bicyclic ketone **11** (326 mg, 23%) as a colorless oil: TLC R_f (benzene/MTBE = 97/3) = 0.48; ^{1}H NMR δ 7.62−7.72 (4H, m), 7.36−7.46 (6H, m), 5.67 (1H, dt, J = 7.6 and 15.2 Hz), 5.06 (1H, dd, J = 6.9 and 15.2 Hz), 4.59 (1H, dt, J = 5.2 and 7.9 Hz), 4.08 (2H, q, J = 7.1 Hz), 2.35 (1H, dd, J = 4.0 and 8.1 Hz), 2.25 (2H, dd, J = 3.6 and 8.4 Hz), 2.20 (2H, t, J = 7.9 Hz), 2.07 (2H, q, J = 6.8 Hz), 1.70−1.80 (2H, m), 1.20−1.50 (11H, m), 1.21 (3H, t, J = 7.1 Hz), 1.05 (9H, s), 0.90 (3H, t, J = 7.1 Hz); ^{13}C NMR δ up 210.7, 173.6, 133.9, 133.6, 60.1, 46.6, 42.2, 34.1, 32.6, 31.3, 29.0, 25.0, 23.7, 22.5, 19.0; down 135.8, 135.5, 134.6, 129.83, 129.80, 127.8, 127.7, 124.8, 67.9, 38.5, 30.3, 26.8, 14.2, 14.1; IR (film) 2930, 1727, 1463, 1428, 1365, 1245, 1154, 1111, 966, 824, 741, 703, 612 cm^{-1}; EI MS m/z (rel intensity) 574 (M^+, 5), 517 (37), 292 (57), 146 (98), 199 (100), 105 (43); EI HRMS calcd for $C_{36}H_{50}O_4Si$ 574.3478, found 574.3474.

Ketones 12a and 12b. To a stirred solution of the bicyclic ketone **3** (620 mg, 1.08 mmol) and thiophenol (0.22 mL, 2.16 mmol) in CH_2Cl_2 (5.4 mL) at −78 °C was added $BF_3{\cdot}OEt_2$ (0.33 mL, 2.70 mmol). After 10 min, the mixture was warmed to −45 °C. After an additional 2.5 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 an inseparable 3:1 mixture of the thioether **12a** and its 6-epimer **12b** (715 mg, 97% yield) as a colorless oil: TLC R_f (petroleum ether/MTBE = 97:3) = 0.61; IR (film) 2930, 2857, 1739, 1463, 1427, 1372, 1175, 1112, 1053, 823, 740, 702, 612 cm^{-1}; FAB MS m/z (rel intensity) 707 (M^+ + Na, 100), 628 (6), 598 (9), 576 (25); FAB HRMS calcd for $C_{42}H_{56}O_4NaSiS$ 707.3566, found 707.3557. Major isomer: ^{1}H NMR δ 7.62−7.67 (12/4H, m), 7.37−7.47 (18/4H, m), 7.17−7.26 (15/4H, m), 5.02−5.16 (6/4H, m), 4.60 (3/4H, d, J = 5.8 Hz), 4.12 (6/4H, q, J = 7.1 Hz), 3.33 (3/4H, dd, J = 3.6, 7.9 Hz), 2.76−2.81 (6/4H, m), 2.51 (3/4H, dd, J = 6.1, 19.6 Hz), 2.24−2.32 (6/4H, m), 1.87−1.96 (3/4H, m), 1.61−1.84 (12/4H, m), 1.41−1.57 (9/4H), 1.25 (9/4H, t, J = 7.1 Hz), 1.16−1.26 (6/4H, m), 1.06−1.12 (12/4H, m), 1.06 (27/4H, s), 0.82 (9/4H, t, J = 7.1 Hz); ^{13}C NMR δ up 217.4, 173.6, 134.6, 133.43, 133.41, 60.2, 46.9, 34.1, 32.0, 31.2, 28.8, 28.0, 25.0, 24.3, 22.4, 19.0; down 135.8, 135.7, 133.3, 132.8, 129.89, 129.86, 128.6, 128.2, 127.8, 127.7, 127.3, 70.6, 52.4, 51.0, 50.2, 26.9, 14.2, 14.0. Minor isomer: ^{1}H NMR δ 7.60−7.70 (4/4H, m), 7.35−7.48 (6/4H, m), 7.18−7.26 (5/4H, m), 5.08 (1/4H, dd, J = 9.6, 15.2 Hz), 4.96 (1/4H, dt, J = 6.7, 15.2 Hz), 4.29 (1/4H, dt, J = 4.7, 6.1 Hz), 4.13 (2/4H, q, J = 7.1 Hz), 3.59 (1/4H, dd, J = 4.9, 9.6 Hz), 2.44 (1/4H, dt, J = 4.7, 6.7 Hz), 2.33 (1/4H, m), 2.31 (2/4H, t, J = 7.6 Hz), 2.16−2.23 (2/4H, m), 1.74−1.82 (4/4H, m), 1.65 (2/4H, quint, J = 6.7 Hz), 1.44−1.53 (1/4H, m), 1.30−1.39 (1/4H, m), 1.25 (3/4H, t, J = 7.1 Hz), 1.15−1.22 (3/4H, m), 0.98−1.12 (4/4H, m), 1.07 (9/4H, s), 0.82 (3/4H, t, J = 7.1 Hz); ^{13}C NMR δ up 217.8, 173.6, 134.7, 133.4, 133.2, 60.2, 47.3, 34.2, 32.0, 31.1, 30.3, 28.7, 26.4, 25.0, 22.4, 19.1; down 135.9, 135.8, 135.3, 132.6, 130.0, 129.9, 128.8, 127.8, 127.7, 127.0, 126.2, 72.5, 53.3, 53.0, 50.0, 26.9, 14.2, 13.9.

Alcohols 13 and 14. To a stirred solution of the inseparable ketone epimers **12a** and **12b** (3:1) (695 mg, 1.02 mmol) in MeOH (14 mL) at 0 °C was added $NaBH_4$. After an additional 1 h, the mixture was partitioned between EtOAc and, seqentially, 5% aqueous HCl, saturated aqueous $NaHCO_3$, and brine. The organic extract was dried (Na_2SO_4) and concentrated. The residue was chromatographed to afford the alcohol from reduction of the trans ketone **12b** (174 mg, 25% yield) as a colorless oil; TLC R_f (petroleum ether/MTBE = 8/2) = 0.44. This was oxidized by the Dess−Martin periodinane to give a quantitiative yield of the ketone **12b**, characterized above. Further elution gave the β-alcohol **14** (208 mg, 30% yield) as a colorless oil: TLC R_f (petroleum ether/MTBE = 8/2) = 0.37; ^{1}H NMR δ 7.64−7.69 (4H, m), 7.35−7.44 (6H, m), 7.20−7.25 (5H, m), 5.25 (1H, dd, J = 9.5, 15.1 Hz), 4.91 (1H, dt, J = 6.9, 15.1 Hz), 4.64 (1H, dt, J = 3.6, 6.1 Hz), 4.21−4.28 (1H, m), 4.12 (2H, q, J = 7.1 Hz), 3.48 (1H, dd, J = 5.2, 9.5 Hz), 2.37−2.48 (3H, m), 2.30 (2H, t, J = 7.5 Hz), 2.05 (1H, ddd, J = 3.6, 6.3, 15.1 Hz), 1.85 (1H, ddd, J = 2.8, 6.3, 15.0 Hz), 1.73−1.80 (2H, m), 1.58−1.68 (2H, m), 1.45−1.58 (2H, m), 1.31−1.38 (1H, m), 1.25 (3H, t, J = 7.1 Hz), 1.17−1.26 (4H, m), 1.07−1.14 (4H, m), 1.05 (9H, s), 0.84 (3H, t, J = 7.1 Hz); ^{13}C NMR δ up 173.8, 134.5, 134.3, 134.0, 60.2, 45.3, 34.2, 32.0, 31.3, 29.0, 28.2, 25.34, 25.27, 22.5, 19.1; down 135.92, 135.86, 133.3, 131.3, 130.4, 129.59, 129.58, 128.6, 127.57, 127.56, 127.4, 76.2, 73.3, 55.1, 52.9, 45.9, 7.0, 14.2, 14.0; IR (film) 3463, 2929, 2856, 1735, 1460, 1427, 1374, 1186, 1112, 1066, 965, 822, 740, 702, 612 cm^{-1}; FAB MS m/z (rel intensity) 709 (M^+ + Na, 90), 687 (34), 679 (76), 673 (62), 671 (59), 643 (35), 629 (100), 627 (95); FAB HRMS calcd for $C_{42}H_{58}O_4SiSNa$ 709.3723, found 709.3747. This was followed by the α-alcohol **13** (313 mg, 45% yield) as a colorless oil: TLC R_f (petroleum ether/MTBE = 8/2) = 0.28; ^{1}H NMR δ 7.69−7.72 (4H, m), 7.35−7.43 (6H, m), 7.17−7.24 (5H, m), 5.16 (1H, dd, J = 9.5, 15.1 Hz), 4.87 (1H, dt, J = 6.9, 15.1 Hz), 4.39 (1H, quint, J = 3.3 Hz), 4.11 (2H, q, J = 7.1 Hz), 3.96−4.03 (1H, m), 3.42 (1H, dd, J = 6.4, 9.5 Hz), 2.60 (1H, dt, J = 3.7, 7.3 Hz), 2.28 (2H, t, J = 7.5 Hz), 2.09−2.25 (1H, m), 2.13 (1H, dt, J = 6.8, 14.7 Hz), 1.56−1.77 (6H, m), 1.24 (3H, t, J = 7.1 Hz), 1.15−1.46 (m, 6H), 1.07 (9H, s), 1.01−1.07 (4H, m), 0.83 (3H, t, J = 7.1 Hz); ^{13}C NMR δ up 173.7, 135.3, 134.3, 133.9, 60.2, 43.8, 34.2, 32.0, 31.2, 28.9, 28.3, 27.9, 25.1, 22.4, 19.1; down 136.00, 135.97, 133.2, 132.0, 129.6, 129.3, 128.5, 127.6, 127.0, 76.9, 76.2, 54.1, 52.4, 50.1, 27.0, 14.2, 14.0; IR (film) 3441, 2929, 2856, 1732, 1634, 1472, 1373, 1257, 1186, 111[1], 102*6*, 822, 740, 703, 612 cm^{-1}; FAB MS m/z (rel intensity) 709 (M^+ + Na, 100), 701 (17); FAB HRMS calcd for $C_{42}H_{58}O_4SiSNa$ 709.3723, found 709.3693.

Conversion of β-Alcohol 14 to α-Alcohol 13. To a stirred solution of the β-alcohol **14** (88 mg, 0.13 mmol) in CH_2Cl_2 (4 mL) at room temperature was added Dess−Martin periodinane (109 mg, 0.26 mmol). After an additional 30 min, the reaction mixture 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 chromatographed to afford the ketone **12a** (88 mg) as a colorless oil. To a stirred solution of the ketone **12a** (44 mg, 0.06 mmol) in MeOH (2 mL) at 0 °C was added $NaBH_4$. After an additional 1 h, the reaction mixture was partitioned between EtOAc and, sequentially, 5% aqueous HCl, saturated aqueous $NaHCO_3$, and brine. The organic extract was dried (Na_2SO_4) and concentrated. The residue was chromatographed to afford the β-alcohol **14** (17.2 mg, 39% recovery). This was followed by the α-alcohol **13** (25.6 mg, 58% yield, 97% based on **14** not recovered).

Diol 15. To a stirred solution of the thioether **13** (142 mg, 0.21 mmol) in CH_2Cl_2 (2.5 mL) at −78 °C was added a solution of *m*-CPBA (77 mg, 0.31 mmol) in CH_2Cl_2 (1.5 mL). The mixture was stirred for 1 h, after which time a solution of trimethyl phosphite (244 μL, 2.07 mmol) in EtOH (2 mL) was added. The mixture was stirred at −78 °C for 5 min and then warmed to room temperature. The reaction mixture was partitioned between EtOAc and, sequentially, 5% aqueous NaOH, saturated aqueous NH_4Cl, and brine. The organic

extract was dried (Na_2SO_4) and concentrated. The residue was chromatographed to afford the diol **15** (116.5 mg, 95% yield) as a colorless oil: TLC R_f (petroleum ether/MTBE = 55/45) = 0.33; ^{1}H NMR δ 7.62−7.67 (4H, m), 7.35−7.45 (6H, m), 5.04−5.15 (2H, m), 4.12 (2H, q, J = 7.1 Hz), 3.99 (1H, quint, J = 2.6 Hz), 3.80−3.88 (2H, m), 2.66 (1H, m), 2.27 (2H, t, J = 7.4 Hz), 2.16−2.23 (2H, m), 2.02 (1H, br s), 1.63−1.77 (2H, m), 1.56−1.62 (2H, m), 1.25 (3H, t, J = 7.1 Hz), 1.19−1.44 (12H, m), 1.07 (9H, s), 0.87 (3H, t, J = 7.1 Hz); ^{13}C NMR δ up 173.8, 134.0, 133.8, 60.2, 43.1, 37.0, 34.2, 31.7, 29.1, 27.7, 25.0, 22.6, 19.0; down 135.9, 135.8, 129.72, 129.68, 128.5, 127.6, 78.8, 77.6, 72.7, 53.9, 50.2, 27.0, 14.2, 14.0; IR (film) 3417, 2930, 2857, 1732, 1463, 1428, 1373, 1258, 1188, 1112, 1066, 970, 823, 741, 703, 613 cm^{-1}; EI MS m/z (rel intensity) 594 (M^+, 1), 520 (33), 519 (68), 303 (51), 199 (100), 135 (26); EI HRMS calcd for $C_{36}H_{54}O_5Si$ 584.3741, found 594.3787.

Triol 16. To a stirred solution of the silyl ether **15** (192 mg, 0.32 mmol) in THF (3 mL) at room temperature was added a 1 M THF solution of n-Bu_4NF (2.59 mL, 2.59 mmol). After 12 h at room temperature, 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 triol **16** (113 mg, 98% yield) as a colorless oil: TLC R_f (EtOAc/MeOH = 95/5) = 0.5; ^{1}H NMR δ 5.53 (1H, dd, J = 7.3, 15.2 Hz), 5.38 (1H, dd, J = 9.7, 15.2 Hz), 4.12 (2H, 2H, q, J = 7.1 Hz), 4.02 (1H, q, J = 6.8 Hz), 3.89−3.97 (2H, m), 3.35−3.85 (1H, br), 2.75−3.30 (2H, br), 2.69−2.74 (1H, m), 2.42 (1H, quint, J = 7.2 Hz), 2.28 (2H, t, J = 7.4 Hz), 2.02−2.09 (1H, m), 1.52−1.64 (4H, m), 1.23−1.48 (11H, m), 1.25 (3H, t, J = 7.1 Hz), 0.89 (3H, t, J = 7.1 Hz); ^{13}C NMR δ up 174.0, 60.3, 42.4, 37.1, 34.1, 31.7, 28.7, 27.5, 25.2, 24.8, 22.6; down 136.1, 129.6, 76.4, 73.1, 53.4, 49.9, 14.2, 14.0; IR (film) 3226. 2925, 2855, 1738, 1463, 1422, 1379, 1305, 1185, 1092, 1035, 976, 730 cm^{-1}; FAB MS m/z (rel intensity) 379 (M^+ + Na, 15), 242 (100), 184 (10), 133 (42), FAB HRMS calcd for $C_{20}H_{36}O_5Na$ 379.2460, found 379.2445.

2,3-Dinor-5,6-dihydro-15F_{2t}-isoprostane (2). To a stirred solution of the ester **16** (20 mg, 0.056 mmol) in THF−H_2O (1:1, 1.5 mL) at room temperature was added LiOH·H_2O (24 mg, 0.56 mmol). After 3 h at room temperature, the reaction mixture at 0 °C was acidified to pH = 4 by adding 1% aqueous HCl (2.2 mL). After the addition of solid NaCl (1 g), the mixture was extracted with $CHCl_3$−MeOH (95:5, v/v). The organic extract was dried (Na_2SO_4) and concentrated. The residue was chromatographed to afford **2** (17 mg, 92% yield) as a white solid: TLC R_f (EtOAc/MeOH/AcOH = 90/10/0.1) = 0.13; ^{1}H NMR (CD_3OD) δ 5.49 (1H, dd, J = 6.6, 15.3 Hz), 5.39 (1H, dd, J = 9.5, 15.3 Hz), 3.98 (1H, q, J = 6.6 Hz), 3.91 (1H, dt, J = 4.2, 6.6 Hz), 3.82 (1H, dt, J = 6.1, 7.6 Hz), 2.60−2.66 (1H, m), 2.46 (1H, m), 2.28 (2H, t, J = 7.4 Hz), 1.98−2.05 (1H, m), 1.27−1.60 (15 H, m), 0.90 (3H, t, J = 6.8 Hz); ^{13}C NMR (CD_3OD) δ up 43.6, 38.4, 33.0, 29.6, 28.7, 26.4, 26.3, 23.7; down 136.8, 130.5, 76.5, 73.8, 54.2, 50.2, 14.4; IR (film) 353, 2929, 2858, 1704, 1462, 1415, 1344, 1260, 1072, 1018, 974, 797 cm^{-1}; FAB MS m/z (rel intensity) 351 (M^+ + Na, 10), 311 (8), 293 (100), 275 (88), 249 (42), 138 (29); FAB HRMS calcd for $C_{18}H_{32}O_5Na$ 351.2147, found 351.2145.

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

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