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Preparation of *ent*-Prostaglandin E_2

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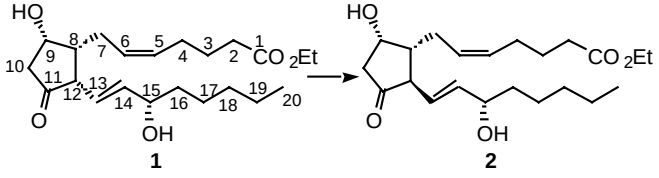
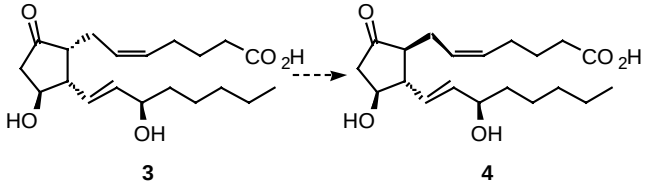
Abstract—The enantiomeric prostaglandins, exemplified by *ent*-PGE_2, are apparently produced in vivo by the nonenzymatic oxidation of arachidonic acid. To assess the physiological activity of *ent*-PGE_2, it was necessary to prepare it by total synthesis. The key transformation in this synthesis was the equilibration of the kinetically prepared *ent*-15-E_{2t}-isoprostane to *ent*-PGE_2.

Preparation of *ent*-Prostaglandin E_2

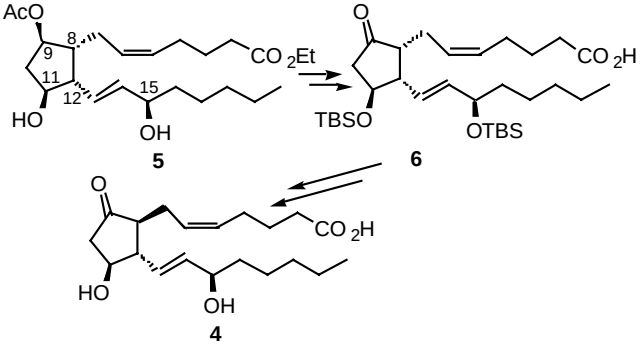
In 1990, Roberts and Morrow reported that a series of prostaglandin-like compounds are produced in vivo in humans independent of the cyclooxygenase enzymes, by free radical-mediated oxidation of membrane-bound arachidonic acid.[1] These oxidation products have been named the isoprostanes (e.g. 15-D_{2c}-isoprostane **1** and *ent*-15-E_{2t}-isoprostane **3**).[2] It is remarkable that although the isoprostanes are not enzymatic products, they do have very potent receptor-mediated physiological activity.[3] The recent observation[4] that the isoprostane **1** is easily epimerized to the prostaglandin **2** raised the likelihood that the isoprostane **3** is epimerized in vivo to the prostaglandin (**4**). It is therefore of interest to assess the physiological activity of **4**.[5] To this end, we have prepared several milligrams of enantiomerically- and diasteromerically-pure *ent*-PGE_2 (**4**).[6]





We planned to prepare **4** from the enantiomerically pure diol **5**[7] (Scheme 1). There were two problems to be solved in effecting this transformation, the selection of the proper protecting groups such that **6** could be efficiently desilylated, and the establishment of conditions to achieve epimerization of **3** to **4** without eliminating the 11-OH.

We first prepared keto acid **6** having the two hydroxy groups protected as *tert*-butyldiphenylsilyl ethers.[5b] Unfortunately, all attempts to desilylate this intermediate with HF·pridine complex or with TBAF led mainly to dehydration. We then turned to protection (Scheme 2) of **5** with TBDMSCl[8] in the presence of imidazole and a catalytic amount of DMAP, to give the acetate **7**. Saponification of **7** with lithium hydroxide in a mixed solvent of THF, water and methanol (1:1:1, v/v) produced the hydroxy acid **8**. Oxidation of **8** with Dess–Martin periodinane[9] provided the keto acid **6**. Desilylation of **6** with HF·pyridine complex[10] in THF and CH_3CN (1:1, v/v) afforded a mixture of *ent*-15-E_{2t}-isoprostane (**3**) and *ent*-PGE_2 (**4**), [**3**:**4**=4.6:1; ^{1}H NMR: **3**: δ 4.28–4.29 (m, 1H), 4.01–4.08 (m, 1H); **4**: δ 4.01–4.08 (m, 0.44H); ^{13}C NMR: Scheme 3], which was further equilibrated[11] with KOAc in methanol at rt to furnish a 93:7



Scheme 1.

Keywords: asymmetric synthesis; biologically active compounds; cyclopentanones; prostanoids.

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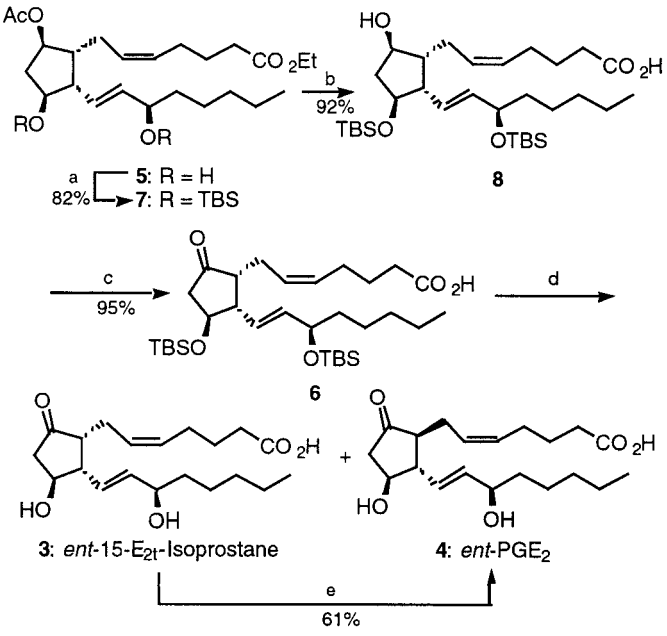
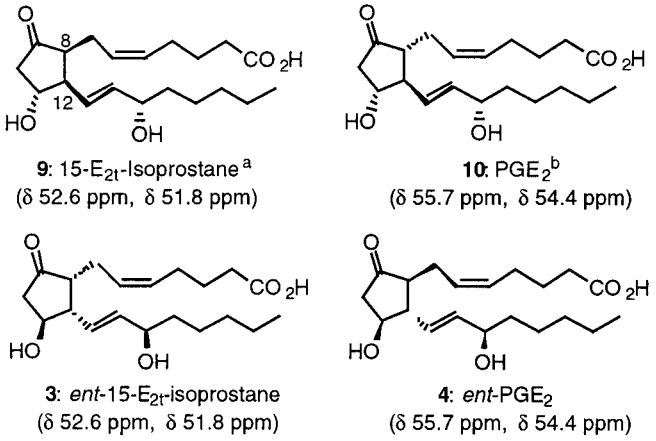


Scheme 2. Key: (a) TBDMSCl, imidazole, DMAP (cat.), CH_2Cl_2, rt; (b) LiOH·H_2O, THF/H_2O/MeOH (1:1:1, v/v), rt; (c) Dess–Martin periodinate, CH_2Cl_2, rt; (d) HF·pyridine, CH_3CN/THF (1:1, v/v), 0°C to rt; (e) KOAc, MeOH, rt.



Scheme 3. [a]Synthetic sample. [b]Commercial sample.

ratio of **4**:**3** which could be separated by column chromatography (EtOAc then EtOAc/MeOH/AcOH=95/5/0.1). The synthetic *ent*-PGE_2 (**4**) was identical with commercial PGE_2 (Sigma) by TLC, MS, IR, ^1H and ^{13}C NMR; $[\alpha]_D=+113.6$ ($c=0.25$, MeOH) [lit.[5] $[\alpha]_D=+80$ (95% EtOH)]. Exposure of commercial PGE_2 to the same equilibration conditions led to a 92:8 ratio of PGE_2:15-E_{2t}-isoprostane.

Detailed studies of the physiological activity of *ent*-PGE_2 (**4**) will be reported elsewhere.

Experimental

General

^1H NMR and ^{13}C NMR spectra were obtained as solutions in deuterochloroform ($CDCl_3$). ^{13}C multiplicities were determined with the aid of a JVERT pulse sequence, differentiating the signals for methyl and methine carbons as 'd', from methylene and quaternary carbons as 'u'. The infrared (IR) spectra were determined as neat oils. Mass spectra (MS) were obtained at an ionizing potential of 15 eV. Substances for which C, H analyses are not reported were purified as specified and gave spectroscopic data consistent with being >95% the assigned structure. Optical rotations were determined as solutions in dichloromethane unless otherwise noted. R_f values indicated refer to thin layer chromatography (TLC) on 2.5×10 cm^2, 250 μm analytical plates coated with silica gel GF, unless otherwise noted, and developed in the solvent system indicated. Column chromatography was carried out with Merck 35–60 mesh silica gel, following the procedure described by Taber.[12] The solvent mixtures used are volume/volume mixtures. All glassware was flame dried under a dry nitrogen stream immediately before use. Tetrahydrofuran (THF), diethyl ether and 1,2-dimethoxyethane (DME) were distilled from

sodium metal/benzophenone ketyl under dry nitrogen. Dichloromethane (CH_2Cl_2) and toluene were distilled from calcium hydride under dry nitrogen. All reaction mixtures stirred magnetically, unless otherwise noted.

Ethyl (5*Z*,8*R*,9*R*,11*S*,12*S*,13*E*,15*R*)-9-acetoxy-11,15-bis-(*tert*-butyldimethylsilyloxy)prosta-5,13-dienoate (7). To a stirred solution of diol **5** (47 mg, 0.11 mmol), imidazole (45 mg, 0.66 mmol), and a catalytic amount of DMAP in dry CH_2Cl_2 (10 mL) was added TBDMSCl (83 mg, 0.55 mmol). The reaction mixture was stirred for 24 h at rt under N_2 and was then partitioned between CH_2Cl_2 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 acetate **7** (59 mg, 82%) as a colorless oil, TLC R_f=0.74 (petroleum ether/MTBE=4/1); $[\alpha]_D$=−14.3 (c=1.48, $CHCl_3$); FAB MS m/z (rel intensity) 675 (M^++Na, 100), 595 (52), 521 (18), 461 (92), 389 (21), 329 (88); IR (film) 2956, 2930, 2857, 1738, 1463, 1362, 1248, 1078, 971, 836, 775 cm^{-1}; ^1H NMR δ 5.49 (dd, 1H, J=6.3 and 15.2 Hz), 5.30–5.40 (m, 2H), 5.25 (dd, 1H, J=9.8 and 15.2 Hz), 4.76 (dt, 1H, J=4.4 and 11.6 Hz), 4.10 (q, 2H, J=7.2 Hz), 4.02 (dd, 1H, J=5.9 and 12.1 Hz), 3.96 (dt, 1H, J=2.9 and 6.0 Hz), 2.60–2.63 (m, 1H), 2.53 (dddd, 1H, J=6.4, 8.4 and 14.8 Hz), 2.35 (quint, 1H, J=7.5 Hz), 2.27 (t, 2H, J=7.5 Hz), 2.02 (s, 3H), 1.92–2.08 (m, 4H), 1.62–1.72 (m, 2H), 1.24–1.55 (m, 9H), 1.24 (t, 3H, J=7.1 Hz), 0.87 (s, 9H), 0.86 (s, 12H), 0.03 (s, 3H), 0.02 (s, 3H), 0.01 (s, 6H); ^{13}C NMR δ up 173.5, 171.1, 60.1, 41.2, 38.4, 33.7, 31.8, 26.7, 26.5, 24.9, 24.8, 22.6, 18.2, 18.0; down 137.0, 129.4, 128.6, 126.6, 78.3, 76.2, 73.3, 53.4, 46.4, 25.8, 25.7, 21.2, 14.2, 14.0, −4.3, −4.70, −4.72, −4.78; FAB HRMS calcd for $C_{36}H_{68}O_6Si_2Na$ 675.4452, found 675.4424.

(5*Z*,8*R*,9*R*,11*S*,12*S*,13*E*,15*R*)-11,15-Bis(*tert*-butyldimethylsilyloxy)-9-hydroxyprosta-5,13-dienoic acid (8). To a stirred solution of acetate **7** (59 mg, 0.09 mmol) in THF/H_2O/MeOH (4.5 mL, 1:1:1, v/v) was added $LiOH\cdot H_2O$ (38 mg, 0.90 mmol). The reaction mixture was stirred for 20 h at rt and was then acidified with 0.5% HCl to pH 4 at 0°C. After the addition of solid NaCl (2 g), the mixture was extracted with $CHCl_3$. The combined organic extract was dried (Na_2SO_4) and concentrated. The residue was chromatographed to yield the hydroxy acid **8** (48.5 mg, 92%) as a colorless oil, TLC R_f=0.37 (EtOAc/MeOH/AcOH=95/5/0.1); $[\alpha]_D$=+14.2 (c=1.62, $CHCl_3$); FAB MS m/z (rel intensity) 605 (M^++Na, 100), 433 (13), 319 (9), 301 (32); IR (film) 3390, 2954, 2929, 2857, 1710, 1463, 1361, 1255, 1079, 1005, 971, 836, 775 cm^{-1}; ^1H NMR δ 5.44–5.52 (m, 2H), 5.35–5.41 (m, 1H), 5.21 (dd, 1H, J=10.0 and 15.2 Hz), 4.03 (dd, 1H, J=5.8 and 11.9 Hz), 3.98–4.01 (m, 1H), 3.86–3.90 (m, 1H), 2.71 (t, 1H, J=8.2 Hz), 2.33 (t, 2H, J=7.4 Hz), 2.26 (dddd, 1H, J=5.3, 7.4, and 14.4 Hz), 2.19 (quint, 1H, J=7.4 Hz), 2.01–2.14 (m, 3H), 1.89–1.96 (m, 1H), 1.63–1.73 (m, 3H), 1.25–1.50 (m, 9H), 0.88 (s, 9H), 0.87 (s, 12H), 0.04 (s, 6H), 0.03 (s, 3H), 0.01 (s, 3H); ^{13}C NMR δ up 178.8, 42.8, 38.4, 33.3, 31.8, 27.3, 26.6, 24.9, 24.5, 22.6, 18.2, 18.0; down 136.5, 129.6, 129.3, 127.2, 78.0, 77.6, 73.3, 54.3, 50.8, 25.9, 25.8, 14.0, −4.3, −4.69, −4.74, −4.8; FAB HRMS calcd for $C_{32}H_{62}O_5Si_2Na$ 605.4034, found 605.4040.

(5*Z*,8*R*,11*S*,12*S*,13*E*,15*R*)-11,15-Bis(*tert*-butyldimethylsilyloxy)-9-oxoprosta-5,13-dienoic acid (6). To a stirred solution of hydroxy acid **8** (48.5 mg, 0.083 mmol) in dry CH_2Cl_2 (10 mL) was added Dess–Martin periodinate (70 mg, 0.17 mmol). The reaction mixture was stirred for 20 h at rt under N_2 and was then chromatographed directly to give the keto acid **6** (46 mg, 95%) as a colorless oil, TLC R_f=0.45 (EtOAc/MeOH/AcOH=95/5/0.1); $[\alpha]_D$=−42.4 (c=1.53, $CHCl_3$); FAB MS m/z (rel. intensity) 603 (M^++Na, 100), 563 (6), 449 (17), 431 (10), 373 (11), 317 (12), 299 (17), 264 (41); IR (film) 2955, 2929, 2857, 1745, 1709, 1458, 1362, 1255, 1070, 1006, 968, 836, 776 cm^{-1}; ^1H NMR δ 5.59 (dd, 1H, J=6.4 and 15.2 Hz), 5.34–5.43 (m, 2H), 5.11 (dd, 1H, J=10.2 and 15.2 Hz), 4.23 (d, 1H, J=5.2 Hz), 4.02 (dd, 1H, J=5.8 and 12.0 Hz), 2.93 (t, 1H, J=8.4 Hz), 2.67–2.72 (m, 1H), 2.46–2.50 (m, 1H), 2.43 (dd, 1H, J=5.2 and 19.0 Hz), 2.33 (t, 2H, J=7.9 Hz), 2.24 (d, 1H, J=19.0 Hz), 2.04–2.13 (m, 2H), 1.80–1.87 (m, 1H), 1.65–1.72 (m, 2H), 1.25–1.49 (m, 8H), 0.87 (s, 21H), 0.08 (s, 3H), 0.06 (s, 3H), 0.02 (s, 3H), 0.00 (s, 3H); ^{13}C NMR δ up 217.7, 179.3, 45.3, 38.2, 33.3, 31.7, 26.6, 24.8, 24.5, 22.9, 22.6, 18.2, 18.0; down 138.1, 129.7, 127.9, 125.5, 73.2, 72.7, 51.9, 50.3, 25.8, 25.7, 14.0, −4.3, −4.7, −4.8, −4.9; FAB HRMS calcd for $C_{32}H_{60}O_5Si_2Na$ 603.3877, found 603.3860.

***ent*-15-E_{2t}-Isoprostane (3) and *ent*-PGE_2 (4).** To a stirred solution of keto acid **6** (23 mg, 0.040 mmol) and pyridine (0.03 mL) in THF/CH_3CN (7 mL, 1:1, v/v) was added 52% HF aqueous solution (0.075 mL) at 0°C. The reaction mixture was warmed to rt. After 6 h stirring at rt, another portion of 52% HF (0.075 mL) was added and stirring was continued for another 18 h. After addition of saturated aqueous NaCl (5 mL) and solid NaCl (0.5 g), the mixture was extracted with $CHCl_3$. The combined organic extract was dried (Na_2SO_4) and concentrated. The residue was chromatographed to furnish a mixture of *ent*-15-E_{2t}-isoprostane **3** and *ent*-PGE_2 **4** (12.5 mg, 90%, **3**:**4**=4.6:1) as a colorless oil.

A solution of KOAc (14 mg, 0.14 mmol) in MeOH (0.5 mL) was added to the mixture of **3** and **4** (12.5 mg, 0.036 mmol, **3**:**4**=4.6:1). The reaction mixture was stirred for 4 days at rt. After addition of saturated aqueous NaCl (5 mL), solid NaCl (0.5 g), and AcOH (0.1 mL), the mixture was extracted with $CHCl_3$. The combined organic extract was dried (Na_2SO_4) and concentrated. The residue was chromatographed to provide *ent*-PGE_2 **4** (8.5 mg, 61% yield from **6**) as a colorless oil.

Partial data for 3. TLC R_f=0.28 (EtOAc/MeOH/AcOH=95/5/0.1); FAB MS m/z (rel. intensity) 375 (M^++Na, 100), 357 (79); IR (film) 3379, 2931, 2856, 1732, 1709, 1398, 1204, 1158, 1120, 1084, 1033, 973 cm^{-1}; ^1H NMR (CD_3OD) δ 5.66 (dd, 1H, J=6.3 and 15.3 Hz), 5.41–5.48 (m, 2H), 5.35 (dddd, 1H, J=0.8, 10.0, and 15.3 Hz), 4.28–4.29 (m, 1H), 4.01–4.08 (m, 1H), 3.04 (dd, 1H, J=8.0 and 9.6 Hz), 2.68–2.73 (m, 1H), 2.58 (dd, 1H, J=5.7 and 19.2 Hz), 2.46 (dt, 1H, J=5.0 and 14.5 Hz), 2.31 (t, 2H, J=7.4 Hz), 2.26 (dd, 1H, J=1.6 and 19.2 Hz), 2.08–2.15 (m, 2H), 1.95–2.03 (m, 1H), 1.64–1.71 (m, 2H), 1.34–1.57 (m, 10H), 0.93 (t, 3H, J=6.2 Hz); ^{13}C NMR (CD_3OD) δ up 219.6, 177.5, 45.6, 38.5, 34.5, 33.1, 27.9,

26.4, 26.1, 24.4, 23.9; down 138.7, 131.1, 129.1, 128.3, 73.4, 72.9, 52.6, 51.8, 14.5.

Data for 4. TLC R_f=0.28 (EtOAc/MeOH/AcOH=95/5/0.1); $[\alpha]_D$=+113.6 (c=0.25, MeOH) [lit.[5a] $[\alpha]_{D=}$+80 (95% EtOH)]; FAB MS m/z (rel. intensity) 375 (M^++Na, 100), 357 (60), 263 (29); IR (film) 3391, 2926, 2857, 1730, 1710, 1408, 1242, 1157, 1116, 1072, 970 cm^{-1}; ^1H NMR (CD_3OD) δ 5.56–5.64 (m, 2H), 5.30–5.45 (m, 2H), 4.01–4.09 (m, 2H), 2.67 (dddd, 1H, J=1.3, 7.4, and 18.3 Hz), 2.30–2.43 (m, 3H), 2.28 (t, 2H, J=7.4 Hz), 2.16–2.22 (m, 1H), 2.04–2.11 (m, 3H), 1.60–1.68 (m, 2H), 1.28–1.56 (m, 10H), 0.91 (t, 3H, J=6.9 Hz); ^{13}C NMR (CD_3OD) δ up 217.1, 177.6, 47.7, 38.5, 34.6, 33.1, 27.9, 26.6, 26.1, 25.9, 23.9; down 137.8, 132.4, 132.0, 128.0, 73.9, 73.0, 55.7, 54.4, 14.6; FAB HRMS calcd for $C_{20}H_{32}O_5Na$ 375.2147, found 375.2166.

Supporting information

^1H NMR and ^{13}C NMR spectra of compounds **4**, **6**, **7**, and **8**. This material is available free of charge via the Internet at http://pubs.acs.org

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