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Syntheses and Preliminary Pharmacological Evaluation of the Two Epimers of the 5-F_{2t}-isoprostane

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Abstract—The total synthesis of the 5-F_{2t}-isoprostane **1** and its 5-epimer **2** from diacetone-D-glucose is described. We report preliminary data on the vascular properties of these compounds.

The isoprostanes are a family of compounds produced in vivo by a free radical peroxidation of arachidonic acid. Depending on which of the labile hydrogen atoms is first abstracted by free radicals, three initial arachidonyl radicals can be formed (Fig. 1), leading to the four prostaglandin H_2-regioisomers. These four compounds can then be fully reduced to form four prostaglandin $F_{2\alpha}$ regioisomers (5-, 8-, 12-, 15-series).

The first demonstration that 15 F_{2t}-isoprostanes were produced in vivo was made by Morrow in 1990.[1] Since that time, 15-F_{2t}-isoprostanes have been extensively quantified as markers of lipid peroxidation in vivo.[2] In addition, 15-F_{2t}-isoprostanes have been shown to possess potent biological activity including vasoconstriction and platelet aggregation,[3] which may be implicated in the pathophysiology of cardiovascular diseases. Aside from 15-F_{2t}-isoprostanes, 5-F_{2t}-isoprostanes have recently been synthesised.[4] Indeed, 5-F_{2t}-isoprostanes appear to be the most abundant identifiable isoprostanes in vivo.[5] However, since these regioisomers are not widely available, their quantification remains limited to a few research groups. Furthermore, these compounds are not available to study their potential biological effects. As a consequence, and in connection with our program directed towards the synthesis of isoprostanes,[6] we synthesised the 5-F_{2t}-isoprostane **1** and its 5(*R*)-epimer **2** from the alkoxyester **3**[7] (Scheme 1) in order to enable the study of their biological properties. We report preliminary data on the vascular properties of these compounds.

Chemistry

The synthesis of 5-F_{2t}-isoprostane **1** and its 5(*R*)-epimer **2**, from the commercially available diacetone-D-glucose as starting material, is shown in Schemes 2 and 3. The first nine steps leading to cyclopentane alkoxyester **3** were achieved in 27% overall yield by using iodo pathway, according to our procedure.[7]

The alkoxyester **3** was converted into the aldehyde **4** by treatment with DIBAL-H in anhydrous toluene with 93% yield (Scheme 2). The introduction of the ω chain of the isoprostane was achieved by using the commercial hexyltriphenyl phosphonium bromide. The aldehyde **4** reacted with the ylide derived from this phosphonium salt and sodium hexamethyldisilyl amide as a base, in anhydrous THF at −80 °C, to afford the pure (*Z*) enic ether **5** in 89% yield.[8] No trace of *trans* compound could be detected by ^{13}C and ^{1}H NMR analysis. All the relative configurations were checked by homonuclear ^{1}H NOE experiments.

Deprotection of the triethylsilyl groups with ammonium fluoride in a mixture of MeOH/THF (2:1) at 60 °C gave the diol **6** in 92% yield. The protection of the hydroxy functions of **6** with benzoyl chloride in dry pyridine gave the colourless diester **7** in 97% yield.

The *tert*-butyldiphenylsilyl ether **7** was converted into the alcohol **8** with a solution of 3% hydrogen chloride[9]

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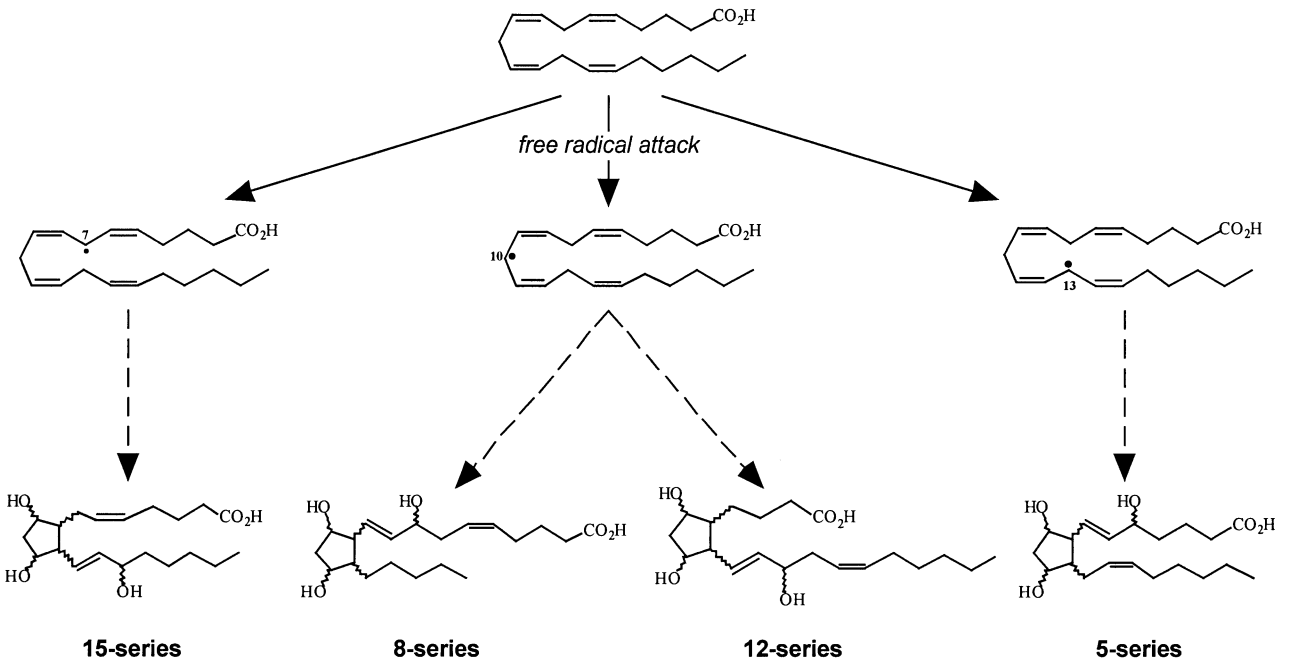
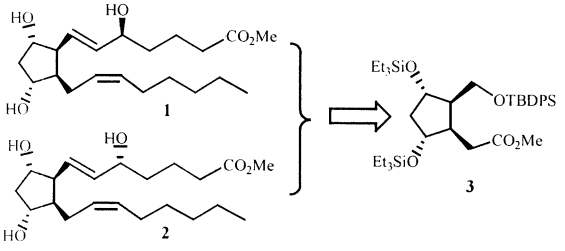


Figure 1.

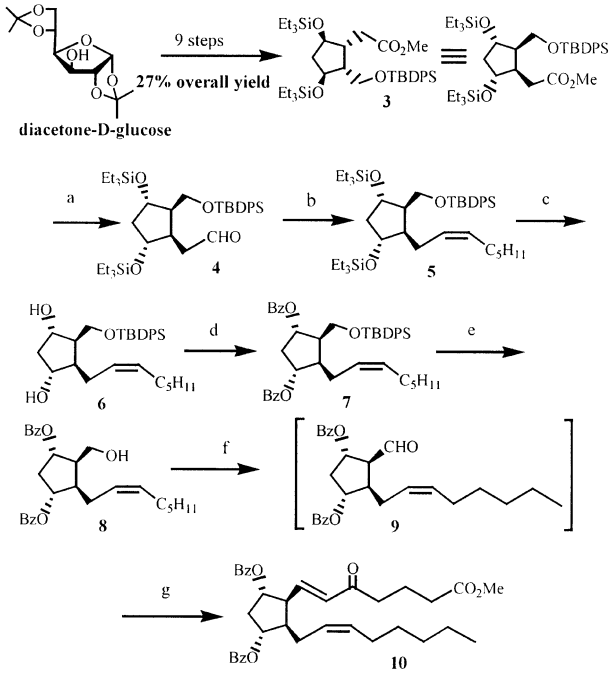


Scheme 1.

in methanol/diethyl ether (1:1, v/v), prepared freshly from acetyl chloride and methanol, a method which proved to be much milder and to give a higher yield (83%) than TBAF in THF. Dess–Martin oxidation[10] of **8** in CH_2Cl_2 gave the unstable aldehyde **9** which was immediately used in the next step without purification. It is important to note that this Dess–Martin oxidation gave higher yield and avoided any epimerization than our first attempts using Swern conditions. The condensation of **9** with diethyl [5-(methoxycarbonyl)-2-oxopentyl]phosphonate,[11] in the presence of sodium hexamethyldisilyl amide, in anhydrous THF at room temperature, afforded the *trans*-α,β-enone ester **10** in 75% overall yield from the alcohol **7** (Scheme 2).

The diastereoselective reduction of the C5 keto group in **10** with the chiral reducing agent[12] (*S*)-BINAL-H proceeded smoothly and gave the desired pure 5(*S*) derivative **11** in 85% yield. Similarly, reduction of **10** using the (*R*)-BINAL-H gave the 5(*R*)-epimer **12** in 87% yield (Scheme 3).

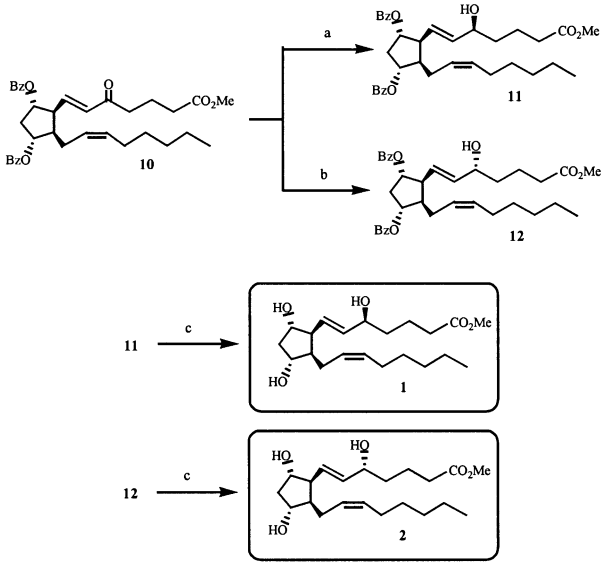
Finally, deprotection of the benzoyl groups in **11** and **12**, with 1 N NaOH at room temperature, followed by excess of CH_2N_2, afforded the desired 5-F_{2t}-isoprostane **1**[13] and its 5-epimer **2**[14] in 93 and 90% yields, respectively.



Scheme 2. (a) 1.1 equiv DIBAL-H (1 M in toluene), toluene, −80 °C, 30 min, 93%; (b) 2.25 equiv hexyltriphenylphosphonium bromide, 2.2 equiv $NaN(SiMe_3)_2$, THF, −80 °C to 20 °C, 2 h, 89%; (c) 4 equiv NH_4F, THF–MeOH, 4 h, 92%; (d) 3 eq. BzCl, pyridine, 20 °C, 1 h, 97%; (e) HCl 3% in MeOH, 20 °C, overnight, 83%; (f) periodinane, CH_2Cl_2, rt, 2 h; (g) 3.3 equiv diethyl[5-(methoxycarbonyl)-oxopentyl]-phosphonate, 3 equiv $NaN(SiMe_3)_2$, THF, 20 °C, 30 min, 75%.

Rat Aorta Preparation

In accordance with the local ethical committee guidelines for animal research, male Wistar rats (270–410 g, IFFA CREDO, Lyon, France) were housed in climate controlled conditions and provided standard rat chow. Animals were anaesthetised with sodium pentobarbital



Scheme 3. (a) (*S*)-BINAL-H, −100 °C, 2 h, 85%; (b) (*R*)-BINAL-H, −100 °C, 2 h, 87%; (c) 1 N NaOH, THF–MeOH, rt, 1 h, then CH_2N_2, 93%.

(Sanofi, Libourne, France, 60 mg kg^{-1} intraperitoneal). Heparin (150 IU, Sanofi Winthrop, Gentilly, France) was injected intravenously. Then, the thoracic aorta was quickly excised, cleaned of connective tissue and cut into 4-mm lengths. Four rings were taken from each thoracic aorta.

Measurement of isometric tension in rings of rat thoracic aorta

The methods used for the measurement of isometric tension in rings of rat thoracic aorta were as previously reported.[15] All the dose–response curves were made by adding increasing concentrations of the agonist to the organ bath in 0.5-log unit steps. The contractile effects of 5-F_{2t}-IsoP and 5-*epi*-5-F_{2t}-IsoP were studied in comparison with U46619, a potent thromboxane A_2/prostaglandin H_2 receptor agonist. Concentration–contraction curves were expressed as percentages of KCl (90 mM)-induced contraction.

Drugs

U46619 (9,11-dideoxy-9α,11α-methanoepoxy-prostaglandin $F_{2\alpha}$), L-phenylephrine, and acetylcholine were purchased from Sigma (Saint Quentin Fallavier, France).

Hydrolysis of methyl ester

The basic hydrolysis of methyl esters of **1** and **2** with aqueous lithium hydroxide in THF at room temperature yielded the desired free acids in 92% yields.

Data analysis

Maximal contraction (E_{max}) and potency (pD_2) were calculated to determine the arterial segment reactivity. The effective concentration of agent that caused 50% of maximal contraction (EC_{50}) was determined from each curve by a logistic, curve-fitting equation. EC_{50} values were expressed as pD_2 ($-\log EC_{50}$). Results are expressed as mean ± SEM.

Results and Discussion

U46619 induced a vasoconstriction in a concentration-dependent manner (pD_2: 7.8 ± 0.1, E_{max}: 189 ± 31%). In contrast, 5-F_{2t}-IsoP and 5-*epi*-5-F_{2t}-IsoP had no effect up to 10^{-5} M ($n = 4$).

5-F_{2t}-IsoP is more abundant than 15-F_{2t}-IsoP in biological fluids in humans.[5] Both compounds are quantifiable in vivo, and currently used as clinical markers of lipid peroxidation in cardiovascular diseases.[3] However, it remains undetermined whether the generation of specific F_2-isoprostane regioisomers differs in clinical situations of increased oxidative stress. Our preliminary observations contrast with data of the 15-series. 15-F_{2t}-IsoP has been shown to be a vasoconstrictor with a potency equivalent to prostaglandin $F_{2\alpha}$, but lower than U46619.[3] Our data suggest that, unlike the 15-series, the 5-series does not possess vascular activity. The 5-series is therefore unlikely to contribute to the pathophysiology of cardiovascular diseases, which could be the major difference between both regioisomers. Such data need to be implemented. The chemical syntheses we described will enable a full pharmacological characterisation of the effects (or the lack of effect) of the 5-F_{2t}-isoprostanes in comparison with 15-F_{2t}-isoprostanes and help to better understand the pharmacological differences between F_2-isoprostane regioisomers.

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8. Compound **5**: ^{1}H NMR (360 MHz, $CDCl_3$) δ ppm 7.64–7.61 (m,4H, H_{Ar}); 7.43–7.24 (m, 6H, H_{Ar}); 5.33–5.29 (m, 2H, H8 and H9); 4.09–4.05 (m, 1H, H4); 3.95–3.89 (m, 1H, H1); 3.59 (d, $J = 5.1$ Hz, 2H, H6); 2.33 (dt, $J = 6.3$ and 13.6 Hz, 1H, H5); 2.20–1.87 (m, 6H, H2, H3, H7 and H10); 1.55–1.43 (m, 1H, H5′); 1.31–1.18 (m, 6H, H11, H12 and H13); 1.05 (s, 9H, H^t_{Bu}); 0.93 (t, $J = 7.9$ Hz, 9H, $SiCH_2CH_3$); 0.87–0.83 (m, 12H, H14 and $SiCH_2CH_3$); 0.56 (q, $J = 7.9$ Hz, 6H, $SiCH_2CH_3$); 0.46 (q, $J = 7.9$ Hz, 6H, $SiCH_2CH_3$). ^{13}C NMR (90 MHz, $CDCl_3$) δ ppm 135.69 (C_{Ar}); 133.54 (C_{Ar}); 130.38 (C9); 129.59

(C_{Ar}); 128.59 (C8); 127.59 (C_{Ar}); 76.41 (C1); 73.26 (C4); 62.64 (C6); 50.68(C3); 48.29 (C2); 45.14 (C5); 31.50 (C12); 29.30 (C11); 27.31 (C10); 26.92 (C_{tBu}); 25.59 (C7); 22.56 (C13); 19.15 ($C(CH_3)_3$); 14.04 (C14); 6.83 ($SiCH_2CH_3$); 6.76 ($SiCH_2CH_3$); 4.93 ($SiCH_2CH_3$); 4.46 ($SiCH_2CH_3$). FAB-MS m/z 709 ($M^{+\cdot}+1$). Anal. calcd for $C_{42}H_{72}O_3Si_3$: C, 71.12; H, 10.23. Found: C, 71.07; H, 10.53.

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13. Compound **1**: UV (ethanol) λ_{max}: 203 nm. ^{1}H NMR (360 MHz, $CDCl_3$) δ ppm 5.62–5.34 (m, 4H, H6, H7, H14 and H15); 4.11–3.95 (m, 3H, H1, H4 and H8); 3.65 (s, 3H, OCH_3); 2.80–2.75 (m, 1H, H3); 2.41 (dt, $J=6.2$, 14.4 Hz, 1H, H5); 2.33 (t, $J=7.1$ Hz, 2H, H11); 2.19–2.08 (m, 1H, H2); 2.02–1.96 (m, 4H, H13 and H16); 1.69–1.51 (m, 5H, H5′, H9 and H10); 1.41–1.24 (m, 6H, H17, H18 and H19); 0.87 (t, $J=6.7$ Hz, 3H, H20). ^{13}C NMR (90 MHz, $CDCl_3$) δ ppm 170.06 (C12); 130.39 (C7); 126.40 (C15); 124.00 (C6); 122.55 (C14); 70.61 (C1 and C4); 67.01 (C8); 48.36 (C3); 46.32 (OCH_3); 45.61 (C2); 37.15 (C5); 31.32 (C9); 28.43 (C11); 26.28 (C18); 24.04 (C17); 22.18 (C13); 21.83 (C16); 17.30 (C19); 15.53 (C10); 8.78 (C20). FAB-MS m/z 369 ($M^{+\cdot}+1$). Anal. calcd for $C_{21}H_{36}O_5$: C, 68.44; H, 9.85. Found: C, 68.38; H, 9.73. IR (NaCl) ν: 3520, 1720.

14. Compound **2**: UV (ethanol) λ_{max}: 203 nm. ^{1}H NMR (360 MHz, $CDCl_3$) δ ppm 5.61–5.33 (m, 4H, H6, H7, H14 and H15); 4.09–3.95 (m, 3H, H1, H4 and H8); 3.66 (s, 3H, OCH_3); 2.78–2.74 (m, 1H, H3); 2.41 (dt, $J=6.1$, 14.1 Hz, 1H, H5); 2.33 (t, $J=7.2$ Hz, 2H, H11); 2.18–2.05 (m, 1H, H2); 2.01–1.93 (m, 4H, H13 and H16); 1.71–1.51 (m, 5H, H5′, H9 and H10); 1.43–1.25 (m, 6H, H17, H18 and H19); 0.87 (t, $J=6.5$ Hz, 3H, H20). ^{13}C NMR (90 MHz, $CDCl_3$) δ ppm 174.06 (C12); 135.64 (C7); 131.60 (C15); 129.60 (C6); 127.75 (C14); 76.36 (C1 and C4); 72.32 (C8); 53.57 (C3); 51.55 (OCH_3); 50.94 (C2); 42.28 (C5); 36.56 (C9); 33.71 (C11); 31.51 (C18); 29.27 (C17); 27.39 (C13); 27.00 (C16); 22.53 (C19); 20.72 (C10); 14.02 (C20). FAB-MS m/z 369 ($M^{+\cdot}+1$). Anal. calcd for $C_{21}H_{36}O_5$: C, 68.44; H, 9.85. Found: C, 68.54; H, 9.87. IR (NaCl) ν: 3520, 1720.

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