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$iPF_{2\alpha}$-III metabolism. First total synthesis of 2,3-dinor $iPF_{2\alpha}$-III, a primary β-oxidation metabolite

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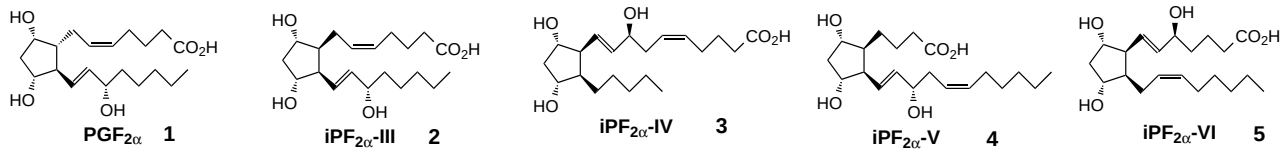
Abstract—The total synthesis of a primary $iPF_{2\alpha}$-III metabolite, namely dinor $iPF_{2\alpha}$-III **8**, is described. The successful synthesis was finally accomplished by generating the carboxylic acid group at the end of the synthesis so as to avoid formation of an α,β-unsaturated carboxylic acid, which is prone to undergo side reactions

Isoprostanes (iPs), a new class of natural products are produced in vivo as a result of free-radical initiated oxygenation of polyunsaturated fatty acids.[1,2] Over the past few years we have performed the first total syntheses of $iPF_{2\alpha}$-III,[3] $iPF_{2\alpha}$-IV,[4] $iPF_{2\alpha}$-V,[5] and $iPF_{2\alpha}$-VI[2] (Scheme 1). Using these synthetic standards, we have discovered the existence of these isoprostanes in biological fluids.[6,7,5,8] We have developed GC–MS[6,7,9] and LC–MS[10,11] methodologies to measure isoprostanes in biological fluids. We have used these sensitive methods to measure increased urinary levels of isoprostane formation and, in particular, $iPF_{2\alpha}$-VI, 8,12-*iso*-$iPF_{2\alpha}$-VI and $iPF_{2\alpha}$-III in disease states such as atherosclerosis[12,2] and Alzheimer's disease[13] and shown that the severity of the diseases correlates with the increase in urinary isoprostane levels.

Before iPs are excreted in urine they undergo a metabolic process. The amount of intact isoprostanes measured in urine represent only a part of the total isoprostanes produced by cells. It is of interest to study the metabolism of these iPs since a metabolite can provide a better index of the isoprostane formation than the original parent.

The metabolism of iPs has not been examined, except for a few published studies with $iPF_{2\alpha}$-III. GC–MS studies have provided evidence for the formation of metabolites such as **6**,[14] **7**,[15] **8**,[16] and **9**[16] (Scheme 2). **6** has attracted the most attention, [14,17] and a synthesis has also been reported.[18] We wish to report here on the first total and stereospecific synthesis of 2,3-dinor $iPF_{2\alpha}$-III metabolite **8**.

We focused on this metabolite because there is a good chance that this is the initial β-oxidation product formed. If this is the case, it will allow us to study its conversion in vivo to **10**, **6** and **9** (Scheme 3). Scheme 3 shows a proposed metabolic sequence to interpret the formation of these products. We propose the intermediacy of the α,β-unsaturated derivative **10** in order to explain the reduction step of **8** to **6**. The transformation sequence shown in the brackets is not unlike earlier work on the metabolism of polyunsaturated fatty



Scheme 1.

Keywords: isoprostanes; $iPF_{2\alpha}$-III; dinor $iPF_{2\alpha}$-III; dinor dihydro-$iPF_{2\alpha}$-III; arachidonic acid.

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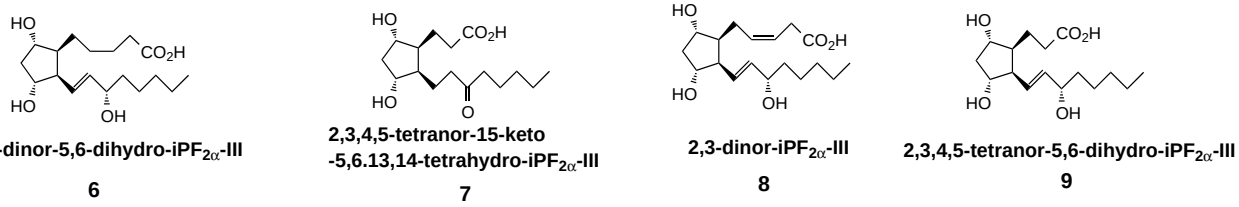
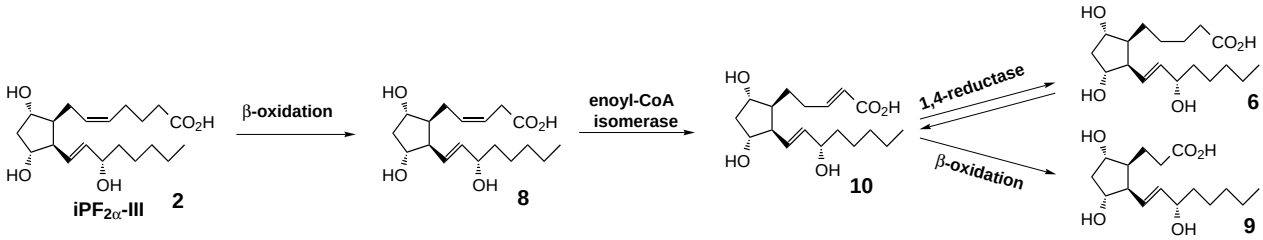


Scheme 2.



Scheme 3.

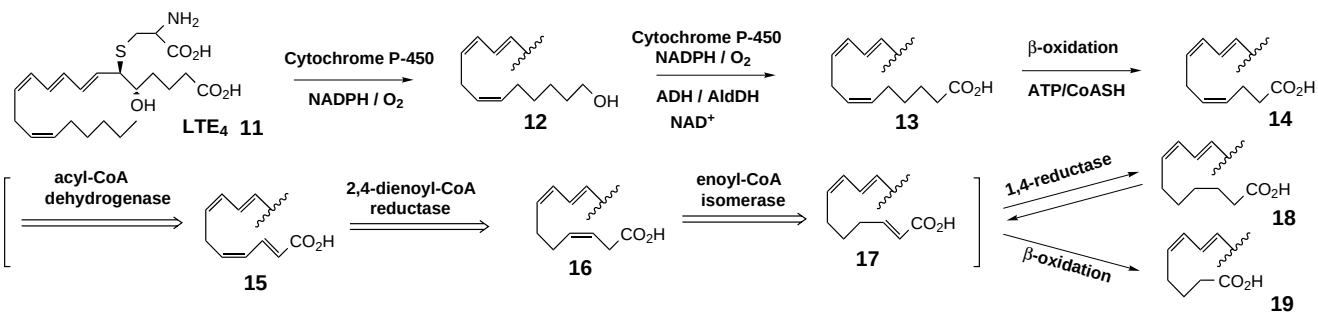
acids[19] and our own work[20] and others[21] on the metabolism of LTE_4 (Scheme 4). Note that the transformation of **16** to **18** and **19** parallels the transformation of **8** to **6** and **9**, as shown in Scheme 3. The formation of **9** can be the result of a β-oxidation cycle from the C-16 derivative **6**, or directly from the α,β-unsaturated derivative **10**. In other words, the α,β-unsaturated derivative **10** is in equilibrium with the reduced form **6**. Hydration of **10**, leading eventually to **9**, will probably draw the equilibrium toward the tetranordihydro derivative **9**. Other metabolic transformations of many of these intermediates, for example oxidation of the hydroxy group to a ketone, are likely also to occur to an unknown degree.[15]

The synthesis of metabolite **8** was performed in 18 steps and is described in Scheme 5. The synthesis of the precyclization synthon **21** was prepared from L-glucose as we reported earlier.[3,22,23]

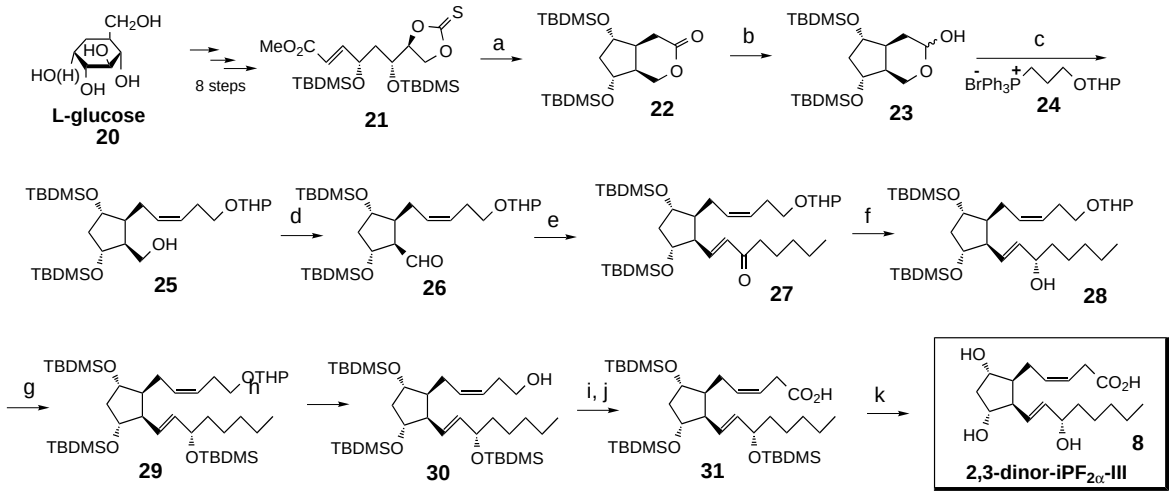
The cyclization of **21** to **22** was performed at reflux in benzene or reflux in toluene. The use of toluene minimizes byproduct formation at the higher temperature by providing a more efficient carbon oxygen homolysis in the radical-generating step. The addition of a three-carbon unit to the top side chain proved difficult. The use of a three-carbon Wittig reagent such as $Ph_3P^{+X-}CH_2CH_2COOH$ and $Ph_3P^{+X-}CH_2CH_2COOCH_3$ was not successful. The use of $Ph_3P^{+X-}CH_2CH_2CH_2O^-$ affords a variable amount of *trans* double bond we would rather avoid.[24] The final design of the synthesis relies on the oxidation of the C-1 alcohol late in the synthesis. The alternative, which was to establish the carboxylic acid and its protected ester earlier, causes migration of the double bond into conjugation with the carboxyl group during the subsequent steps of the synthesis, resulting in mixtures which are difficult to handle. The *S*-BINAL-H reduction of the carbonyl derivative **27** to afford **28** worked in 96% e.e. and in excellent yield. We found that the deprotection of the three TBDMS groups in **31**[25] to yield the target metabolite **8**[26] was best achieved using formic acid.

A word of caution on the metabolism of $iPF_{2\alpha}$-III: as mentioned earlier, $iPF_{2\alpha}$-III produces a dinor dihydro metabolite **6** which appears more abundant than the parent $iPF_{2\alpha}$-III.[14,16] As we have shown in a recent report,[17] γ-linolenic acid **32**, which is a C-18 PUFA with three conjugated double bonds, on free radical peroxidation in vitro gives rise to an isoprostane identical to **6** (Scheme 6).

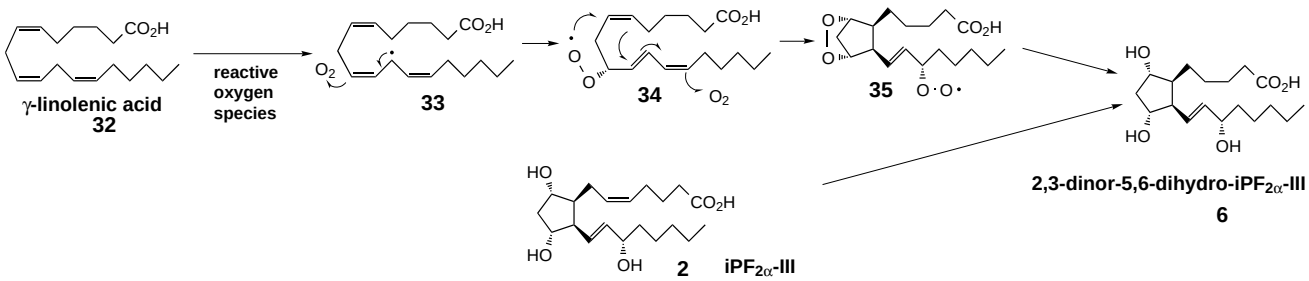
The experiment we did which precludes metabolism is as follows. A blood sample was subjected to a freeze, thawing process in order to allow a peroxidation process. Under these conditions a substantial amount of **6**



Scheme 4.



Scheme 5. *Reagents and conditions*: (a) n-Bu_3SnH, AIBN, benzene, reflux, 5 h, 52%; (b) DIBAL-H, CH_2Cl_2, −78°C, 97%; (c) t-BuOK, THF, −20°C, 5 h, 85%; (d) periodinane, CH_2Cl_2, rt, 2 h, 97%; (e) $(Ome)_2POCH_2CO(CH_2)_4CH_3$, LiHMDS, THF, −78°C, 91%; (f) S-BINAL-H, THF, −100°C, 4 h, 96%; (g) TBDMSCl, IM, CH_2Cl_2, rt, 8 h, 99%; (h) Me_2AlCl, CH_2Cl_2, −20°C to rt, 3 h, 96%; (i) periodinane, t-BuOH, CH_2Cl_2, rt, 3 h, 97%; (j) $NaClO_2$, KH_2PO_4, t-BuOH, 2-methyl-2-butene, H_2O, rt, 96%; (k) 6:3:1 = THF:formic acid:H_2O, rt, 3 h, 78%.



Scheme 6.

was formed. This indicates to us that the measurement of metabolite **6**, as an indicator of $iPF_{2\alpha}$-III production, may be augmented with the peroxidation product of γ-linolenic acid **32**. The measurement of metabolite **8**, the synthesis of which is described in this paper, may be a better indicator of $iPF_{2\alpha}$-III metabolism. Clearly more work is necessary in order to substantiate this idea.

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25. NMR data for compound **31**: ^{1}H NMR (360 MHz, $CDCl_3$) δ 5.59 (m, 2H), 5.46 (dd, $J=5.4$ and 15.4 Hz, 1H), 5.29 (dd, $J=6.1$ and 15.5 Hz, 1H), 4.07 (q, $J=5.1$ and 5.8 Hz, 1H), 3.92 (m, 1H), 3.79 (q, $J=7.4$, 1H), 3.12 (d, $J=6.3$ Hz, 2H), 2.54 (t, $J=6.8$ Hz, 1H), 2.37 (m, $J=6.5$, 6.9 and 7.9 Hz, 1H), 2.15 (m, 2H), 2.09-1.81 (m, 3H), 1.73-1.08 (m, 6H), 0.89 (m, 30H), 0.05 (m, $J=6.5$ and 8.4 Hz, 18H).
26. The NMR and mass spec data of compound **8**: ^{1}H NMR (360 MHz, d_6-acetone) δ 5.57 (m, 2H), 5.50 (m, 2H), 4.01 (q, $J=5.0$ Hz, 1H), 3.94 (m, $J=2.9$ and 3.6 Hz, 1H), 3.88 (q, $J=6.3$, 1H), 3.06 (br, 2H), 2.65 (m, 1H), 2.41(m, $J=7.1$, 7.2, and 14.3 Hz, 1H), 2.16 (m, 2H), 1.59–1.22 (m, 6H), 0.87 (t, $J=6.3$, 3H). MS(NCI), PFB, TMS deriv. m/z 541. MS(EI) methyl ester, TMS deriv. m/z 556 (M^+), 541 (M–15), 485 (M–71), 466 (M–90), 443 (M–113), 395 (M–90–71) base peak, 353 (M–113–90), 305 (M–90–90–71).