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iPF$_{2\alpha}$-III-17,18,19,20-d_4: Total synthesis and metabolism

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Abstract—The first total synthesis of 17,18,19,20-d_4-iPF$_{2\alpha}$-III **32**, a deuterated analog of iPF$_{2\alpha}$-III, is described. We have used this analog in some β-oxidation studies with rat liver homogenates and have shown that **32** was metabolized to 17,18,19,20-tetradeutero-2,3-dinor-iPF$_{2\alpha}$-III **36** and 17,18,19,20-tetradeutero-2,3-dinor-5,6-dihydro-iPF$_{2\alpha}$-III **37**.
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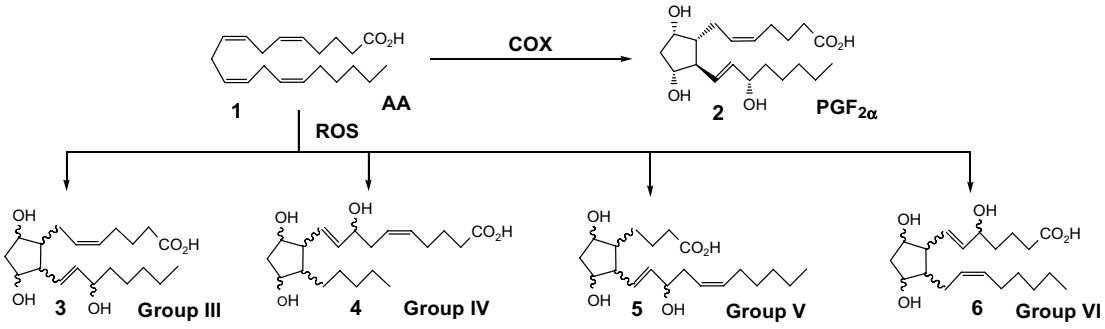
Recently a new class of naturally occurring lipids, the isoprostanes (iPs), has been discovered.[1–3] These molecules are isomeric with prostaglandins. Whereas prostaglandins are formed by the action of cyclooxygenase on arachidonic acid (AA), iPs are produced nonenzymatically by a free-radical peroxidation of polyunsaturated fatty acids such as AA. These iPs, formed in situ on phospholipids, are subsequently released in the free form by the action of phospholipases. Free radical peroxidation of AA affords four groups of iPs (III, IV, V, VI) and each group contains 16 stereoisomers (Scheme 1).[4] We have introduced a formula by which we can calculate the number of iP groups for any given PUFAs as a function of the number of skipped double bonds: $N = (n - 2) \times 2$, where N is the number of iP groups formed, and n the number of skipped double bonds.[5] For example, the number of iP groups derived from eicosapentanoic acid (EPA) is $N = (5 - 2) \times 2 = 6$. These iPs, in particular the more abundant Group VI iPs, have been used as a marker of oxidative stress in disease states such as atherosclerosis[6] and Alzheimer's.[7] Some iPs, in particular Group III, have potent biological effects, mainly due to their interaction with the receptors of the enzymatically generated prostanoids.[8–10] We have



Scheme 1. Groups of isoprostanes derived from AA.

Keywords: iPF$_{2\alpha}$-III; d_4-iPF$_{2\alpha}$-III; Dinor-iPF$_{2\alpha}$-III; Dinor-dihydro-iPF$_{2\alpha}$-III; Arachidonic acid, Metabolism.
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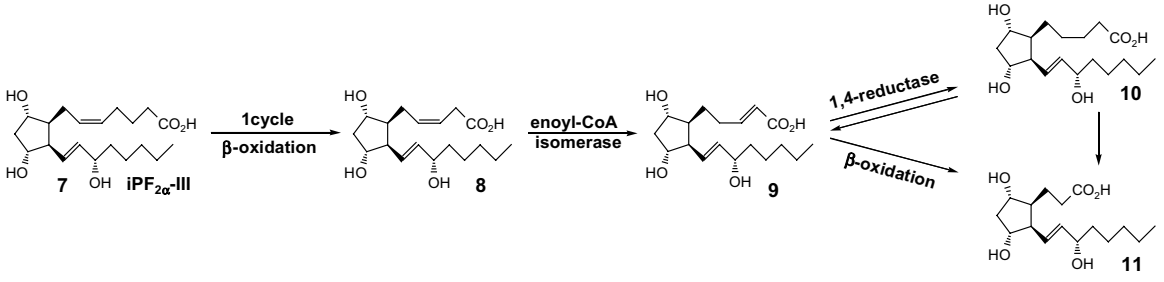
proposed a nomenclature of iPs which is used in the present article.[4] Another nomenclature of iPs has also been reported.[11]

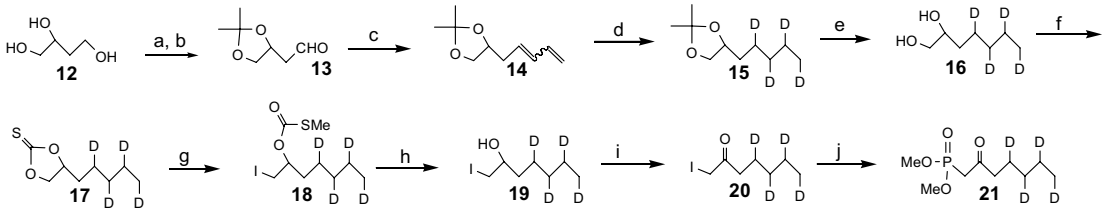
Because of the large number of isomeric iPs, a method of choice for their analysis in biological fluids has been mass spectrometry. The stereospecific chemical synthesis of these compounds has been the only way to obtain pure standards of known stereochemistry that can be used for mass spectrometric analysis and in metabolic studies, as well as for biological evaluation. We have previously reported the total syntheses of several of iPs and have shown that identical products are formed in vivo.[3,12,13] We have also prepared the corresponding deuterium-labeled analogs of some of these compounds to serve as markers for identification and quantitation by mass spectrometry.[14,15] iPs, formed in vivo, are metabolized before they are excreted in urine. $iPF_{2\alpha}$-III is the only iP for which some metabolic studies have been reported. It has been shown that this substance is metabolized by β-oxidation to 2,3-dinor (**8**), 2,3-dinor-5,6-dihydro (**10**), and tetranor (**11**) metabolites as shown in Scheme 2.[16–19] The proposed metabolic pathway shown in Scheme 2 is based on work that we[20] and others[21,22] did on the metabolism of LTE_4 and on the re-examination of the mechanism of the metabolism of unsaturated fatty acids.[23] The objectives of the present study were the following: (a) to synthesize a deuterium analog of $iPF_{2\alpha}$-III that can be used as a quantitative marker for the mass spectrometric analysis of this iP in biological fluids; (b) to select a deuterium-labeled analog of $iPF_{2\alpha}$-III that would be resistant to loss of the deuterium atoms as a result of β-oxidation and therefore useful for metabolic studies; (c) to carry out a preliminary in vitro study of one cycle of β-oxidation; and lastly, (d) to prepare enzymatically the tetradeuterated analogs of β-oxidation metabolites **8** and **10** from the synthetic tetradeutero-$iPF_{2\alpha}$-III **32**. These could be used in further metabolic studies and hence save considerable time for a separate total synthesis of these deuterated metabolites at this particular juncture.

1. Synthesis of 17,18,19,20-d_4-$iPF_{2\alpha}$-III

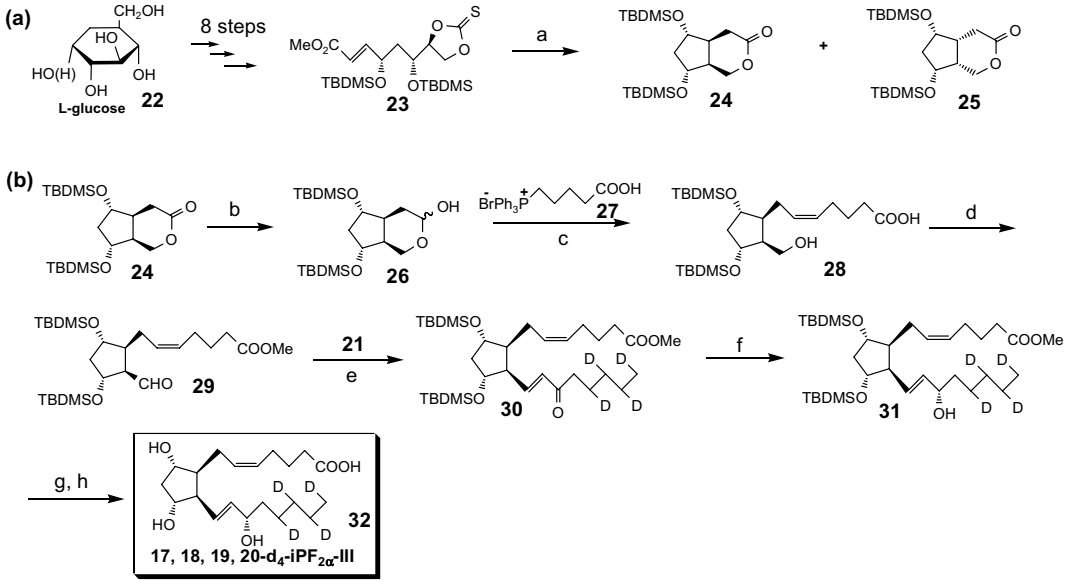
We have designed the synthesis of tetradeuterated analog of $iPF_{2\alpha}$-III in such a way that the deuterium atoms were introduced at positions 17,18,19, and 20. Such a molecule (17,18,19,20-d_4-$iPF_{2\alpha}$-III **32**) would be more resistant to loss of the deuterium atoms as a result of β-oxidation. Scheme 4 shows the convergent synthesis of 17,18,19,20-d_4-$iPF_{2\alpha}$-III **32**, making use of **21** and **24** as key synthons. β-keto phosphonate **21** was synthesized from 1,2,4-butanetriol **12** as shown in Scheme 3. Aldehyde **13** was prepared from 1,2,4-butanetriol **12** in two steps. The Wittig reaction of **13** with commercially available allyltriphenylphosphonium bromide in THF with lithium hexamethyldisilazane as base afforded *cis* and *trans* mixtures of **14** in 2:3 ratio. Using the Wilkinson catalyst, the deuteration of **14** in benzene afforded **15** with a very high incorporation of D_2 (greater than 99%). After removal of the isopropylidene group in **15** with 4% aqueous H_2SO_4, the resulting 6,7-dihydroxy compound **16** was treated with thiocarbonyl bis (imidazole) in acetonitrile to afford **17** in 94% yield. The conversion of thionocarbonate **17** to iodohydrin **19** was performed following a methodology described by us recently.[24] Treatment of **17** with methyl iodide in



Scheme 2. Proposed metabolism pathway for the formation of **8**, **10**, and **11**.



Scheme 3. Reagents and conditions: (a) 2,2-dimethoxypropane, TsOH, CH_3CN, 1 h, 65%; (b) PCC, camphorsulfonic acid, CH_2Cl_2, 3 h, 74%; (c) $H_2C{=}CHCH_2P(C_6H_5)_3Br$, LiHMDS, −78 °C to rt, 2 h, 89%; (d) Wilkinson's catalyst, 99.98% D_2, benzene, 8 h, 87%; (e) 4% H_2SO_4, THF, 8 h, 99%; (f) Im_2CS, CH_3CN, 94%; (g) CH_3I, $ClCH_2CH_2Cl$, 70 °C, 10 h, 86%; (h) DIBAL-H, CH_2Cl_2, −78 °C, 40 min, 96%; (i) Jones reagent, acetone, 0 °C; (j) $P(OMe)_3$, THF, 45 °C, 10 h, 69% (two steps).



Scheme 4. Reagents and conditions: (a) *n*-Bu_3SnH, AIBN, benzene, reflux, 5 h, 52%; (b) DIBAL-H, CH_2Cl_2, −78 °C, 87%; (c) *t*-BuOK, HMPA, THF, −78 °C to rt, 1 h, 68%; (d) periodinane, CH_2Cl_2, rt, 8 h; (e) NaHMDS, THF, −78 °C to rt, 72%; (f) (*S*)-BINAL-H, THF, −100 °C, 4 h, 99%; (g) THF:formic acid:H_2O—6:3:1, rt, 8 h; (h) 5% KOH, THF, 0 °C to rt, 3 h, 76% (two steps).

1,2-dichloroethane at reflux gave **18** in 86% yield. The reduction of **18** with DIBAL-H, followed by an acidic work up furnished **19** in 97% yield. The β-keto iodide **20** derived from the Jones oxidation of **19** was treated with trimethylphosphite in THF at room temperature affording β-keto phosphonate **21** in 62% yield (two steps).

Intermediate **24**, which was prepared from L-glucose in 9 steps,[12] was reduced by DIBAL-H in 87% yield. The resulting lactol **26** was coupled with phosphorane derived from the phosphonium salt **27** by a Wittig reaction to afford **28** in 68% yield. Dess–Martin oxidation of **28** gave **29** which was then used in a Horner–Emmons reaction at −78 °C to introduce the lower side chain using the anion of β-ketophosphonate **21** (prepared following Scheme 3) afforded **30** in 72% yield. The enantioselective reduction of the C-15 keto group in **30** with chiral reducing agent (*S*)-BINAL-H proceeded well and afforded the desired pure **15**(*S*) derivative **31** in 99% yield (ee ~ 98%). The deprotection of bis-silyl groups in **31** was carried out using formic acid. Finally, hydrolysis with aqueous KOH afforded the desired d_4-$iPF_{2\alpha}$-III **32**.[25]

2. Metabolism and MS studies

To determine whether 17,18,19,20-tetradeutero-$iPF_{2\alpha}$-III **32** would be resistant to the loss of deuterium atoms as a result of β-oxidation and therefore useful for metabolic studies, **32** was incubated with rat liver homogenates as described by us earlier.[26] The metabolites that were formed were extracted and analyzed by GC/MS. We also have decided to include in these metabolic studies the commercially available deuterated analog of $iPF_{2\alpha}$-III, 3,3,4,4-tetradeutero-$iPF_{2\alpha}$-III **33**, which has the deuterium atoms at positions 3 and 4.[27] The results of these experiments are summarized in Table 1.

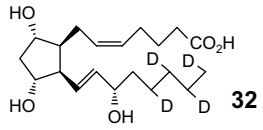
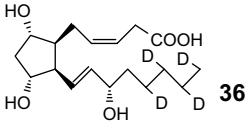
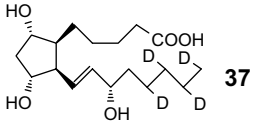
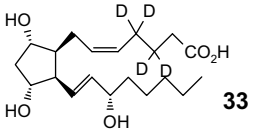
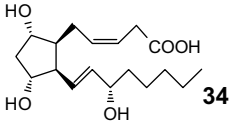
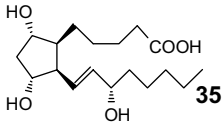
3. Results and discussion

$iPF_{2\alpha}$-III (i.e., 8-*iso*-$PGF_{2\alpha}$) **7**, a Group III iP, was the first iP identified in urine[1] and we have previously performed the first total synthesis of this iP.[12] For quantitative analysis of $iPF_{2\alpha}$-III, we have used its [$^{18}O_2$] analog.[28] We have described here the first total synthesis of an isotopically labeled analog of $iPF_{2\alpha}$-III (**32**) in which the ω-side chain has been labeled with four atoms of deuterium.

$iPF_{2\alpha}$-III is the only isoprostane for which some metabolic studies have been described in any detail. The major urinary metabolite formed after administration of tritium-labeled $iPF_{2\alpha}$-III to humans was identified by gas chromatography–mass spectrometry as 2,3-dinor-5,6-dihydro-$iPF_{2\alpha}$-III.[16] There is also evidence for the formation of 2,3-dinor-$iPF_{2\alpha}$-III in humans and rats.[17,18] In rabbits, the major radiolabeled urinary metabolites identified following administration of tritium-labeled $iPF_{2\alpha}$-III was 5,6-dihydro-2,3,4,5-tetranor-15-keto-13,14-dihydro-$iPF_{2\alpha}$-III.[19] The 17,18,19,20-d_4 analog of $iPF_{2\alpha}$-III reported in the present study would be very useful for studies on the turnover of these metabolites in vivo, as none of the deuterium atoms would be lost, in contrast to the tetradeutero **33** where we expect some loss of deuterium.

The experimental results (Table 1) bear this out. Incubation of **32** with rat liver homogenates, under the conditions briefly described in the footnotes of Table 1, resulted in the formation of two main compounds, the 17,18,19,20-tetradeutero-2,3-dinor-$iPF_{2\alpha}$-III **36** and 17,18,19,20-tetradeutero-2,3-dinor-5,6-dihydro-$iPF_{2\alpha}$-III **37**. Under the same conditions, incubation of **33** yielded exclusively the non-deuterated metabolites, namely 2,3-dinor-iPF_2-III **34** and 2,3-dinor-5,6-dihydro-$iPF_{2\alpha}$-III **35**. It is interesting to note that the enzymatic

Table 1. Metabolites formed from deuterated analogs of $iPF_{2\alpha}$-III

Substrate[a,b]	Metabolites	% Conversion
 **32** **17,18,19,20-tetradeutero-$iPF_{2\alpha}$-III**	 **36** **17,18,19,20-tetradeutero-2,3-dinor-$iPF_{2\alpha}$-III**	3.6
	 **37** **17,18,19,20-tetradeutero-2,3-dinor-5,6-dihydro-$iPF_{2\alpha}$-III**	2.2
 **33** **3,3,4,4-tetradeutero-$iPF_{2\alpha}$-III**	 **34** **2,3-dinor-$iPF_{2\alpha}$-III**	0.2
	 **35** **2,3-dinor-5,6-dihydro-$iPF_{2\alpha}$-III**	0.03

[a] The substrate (20 μM; either **32** or **33**) was incubated with rat liver homogenates in the presence of ATP, CoA, L-carnitine, and NAD+ for 90 min at 37 °C. The incubation was quenched by addition of MeOH and the resulting mixture was centrifuged. The metabolites were isolated from the supernatant by SPE following a methodology described by us previously.

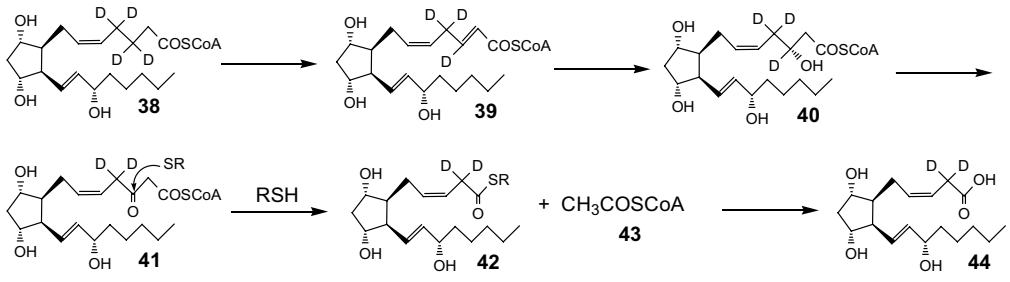
[b] The metabolites were derivatized and analyzed by GC/MS as described by us previously. Briefly, the metabolites were esterified with pentafluorobenzyl bromide in the presence of DIPEA using CH_3CN as solvent. The resulting esters were purified by TLC and derivatized in pyridine by using bis(trimethylsilyl) trifluoroacetamide. The mixture was dried under N_2 and was redissolved in dodecane for GC/MS analysis. A Hewlett-Packard 5973 mass spectrometer interfaced with a 6890 gas chromatograph and a 7683 autosampler was used for analysis.

β-oxidation of **33** is much slower than that of **32**, a fact we attribute to the deuterium isotope effect in **33**, which generally slows down the rate of reaction.

The β-oxidation of 3,3,4,4-tetradeutero- $iPF_{2\alpha}$-III merits some comments. A look at the accepted metabolic sequence for β-oxidation as applied to this 3,3,4,4-tetradeutero derivative **33** is shown in Scheme 5.

Instead of the anticipated dideutero metabolite **44**, we obtained the non-deuterated metabolite, 2,3-dinor-$iPF_{2\alpha}$-III **34**. This was somewhat surprising. A look at the β-oxidation sequence shown in Scheme 5 indicates that biosynthetic intermediate **41**, or possibly **42**, are activated by carbonyl functions and are the most likely candidates in which the deuterium could have been exchanged with hydrogen. To our knowledge this is the first time such an observation has been made in the isoprostane field.[29]

The availability of synthetic **32** in addition to its use as a quantitative marker will allow the enzymatic preparation and use of **36** and **37** in further stepwise metabolic studies. We have previously prepared [$^{18}O_2$] analog of **10** (non-deuterated analog of **37**), which we have used as a marker in our MS studies.[17] While the use of [$^{18}O_2$] derivatives is extremely helpful, the preparation[30] is sometimes tedious and time consuming. Tetradeuterated



Scheme 5.

metabolite **37**, prepared as described in Table 1, would be a good replacement to the [$^{18}O_2$] analog. **36** and **37** separate very easily by HPLC (retention time: 12.9 min (**36**) and 14.6 min (**37**).[31] In addition, since metabolite **10** has been found to be more abundant in urine than its parent compound, its measurement in biological fluids with **37** as marker could be a better index of oxidative stress than iPF$_{2\alpha}$-III.

The spread of the deuterium arrangement in **32** could provide us with additional advantages for the study of iPF$_{2\alpha}$-III metabolism. For example, ω-oxidation which is known to occur in prostaglandins, will transform the ω-carbon into a hydroxy and carboxy derivative. The product of such a metabolic step, in the case of **32**, would be a molecule with two or three deuteriums at 17, 18 and/or 19, which would be enough to identify and quantitate the initial metabolism at C-20.

Acknowledgements

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25. ^{1}H NMR (360 MHz, CD_3OD) δ 5.51–5.58 (m, 4H), 3.93 (m, 2H), 3.81 (q, 1H, J = 6.3 Hz), 3.06 (br, 2H), 2.62 (m, 1H), 2.42 (m, 1H, J = 7.0, 6.8 and 14.1 Hz), 2.23 (t, 2H, J = 7.3), 2.12–1.91 (m, 4H), 1.64–1.18 (m, 9H), 0.82 (t, 2H); MS (EI) (*m*/*z*) 357.6 [M^+], 339 [M − 18], 313 [M − 44], 251, 197, 193 (base peak).
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31. HyperClone C18-BDS, 5 μm, 150 × 2 mm column; mobile phase: H_2O:CH_3COOH (solvent A) and CH_3CN:MeOH:CH_3COOH (solvent B).