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Eicosapentaenoic-acid-derived isoprostanes: Synthesis and discovery of two major isoprostanes

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ABSTRACT

The stereospecific synthesis of two all-*syn*-EPA-derived isoprostanes (iPs), 5-*epi*-8,12-*iso*-iPF$_{3\alpha}$-VI **17** and 8,12-*iso*-iPF$_{3\alpha}$-VI **18**, has been accomplished. These two synthetic probes have been used to discover and identify their presence in human urine. The eventual quantitative measurement of these two iPs may be a valuable index of oxidative stress in people with eicosapentaenoic acid- (EPA) and docosahexaenoic acid- (DHA) enriched phospholipids.

Isoprostanes (iPs) are natural products, isomeric with prostaglandins (PGs). Unlike PGs they are produced non-enzymatically by a free-radical oxygenation of arachidonic acid (AA), a polyunsaturated fatty acid[1,2] (Scheme 1), mostly esterified to phospholipids. The mechanism of iP formation has been discussed in some detail.[3–5]

The formation of these oxygenation products has been related to oxidant stress and inflammatory diseases such as atherosclerosis[6] and Alzheimer's.[2,7] Whereas a substantial amount of work has been focused on AA-generated iPs, little attention has been given to eicosapentaenoic-acid- (EPA) derived iPs.[8,9] We are reporting here on the synthesis and identification of two major all-*syn*-EPA-derived iPs, namely 5-*epi*-8,12-*iso*-iPF$_{3\alpha}$-VI **17** and 8,12-*iso*-iPF$_{3\alpha}$-VI **18** (Scheme 2).

The selection of EPA-derived iPs as our target focus stems from the following: EPA, as well as docosahexaenoic acid (DHA), are widely used as dietary supplements under the label of omega-3 fish oil. Various foods containing increased ratios of omega-3 to omega-6 polyunsaturated fatty acids, such as milk and eggs, are also widely available. In addition, there are some sectors of the population with high EPA and DHA intake through their high fatty fish diets, for example, Inuits, etc. The rationale for their use is to enrich the phospholipids with EPA and DHA and reduce the percentage of other polyunsaturated fatty acids, especially arachidonic acid. EPA and DHA are somewhat poorer substrates for cyclooxygenase than AA and are also partial enzyme inhibitors, with one result being the diminution of TXA_2 production and reduced platelet aggregation. This may lead to reduced cardiovascular incidents such as heart attacks.

The AA-derived iPs we and others have measured so far as an index of oxidant stress[10,11] may not be representative for people in which the major polyunsaturated fatty acids in the phospholipids are EPA and DHA. A more appropriate index in those cases might be the measurement of EPA-derived iPs, for example, **17** and **18**.

Target selection: The reason that we were particularly interested in **17** and **18** stems from the hypothesis that 5-hydroxy-isoprostanes are resistant to β-oxidation.[8] As shown in Scheme 1, of the six iP groups, only Group VI **9** has a hydroxyl group in the 5-position. We and others have proposed in the past that such a relationship is responsible for the lack of metabolism by β-oxidation at C-1 of other eicosanoids including LTE_4 and LTB_4[8,12] possibly due to complexation and inactivation of the β-oxidation enzymes. We extended that concept to the iP field and hypothesized that if such

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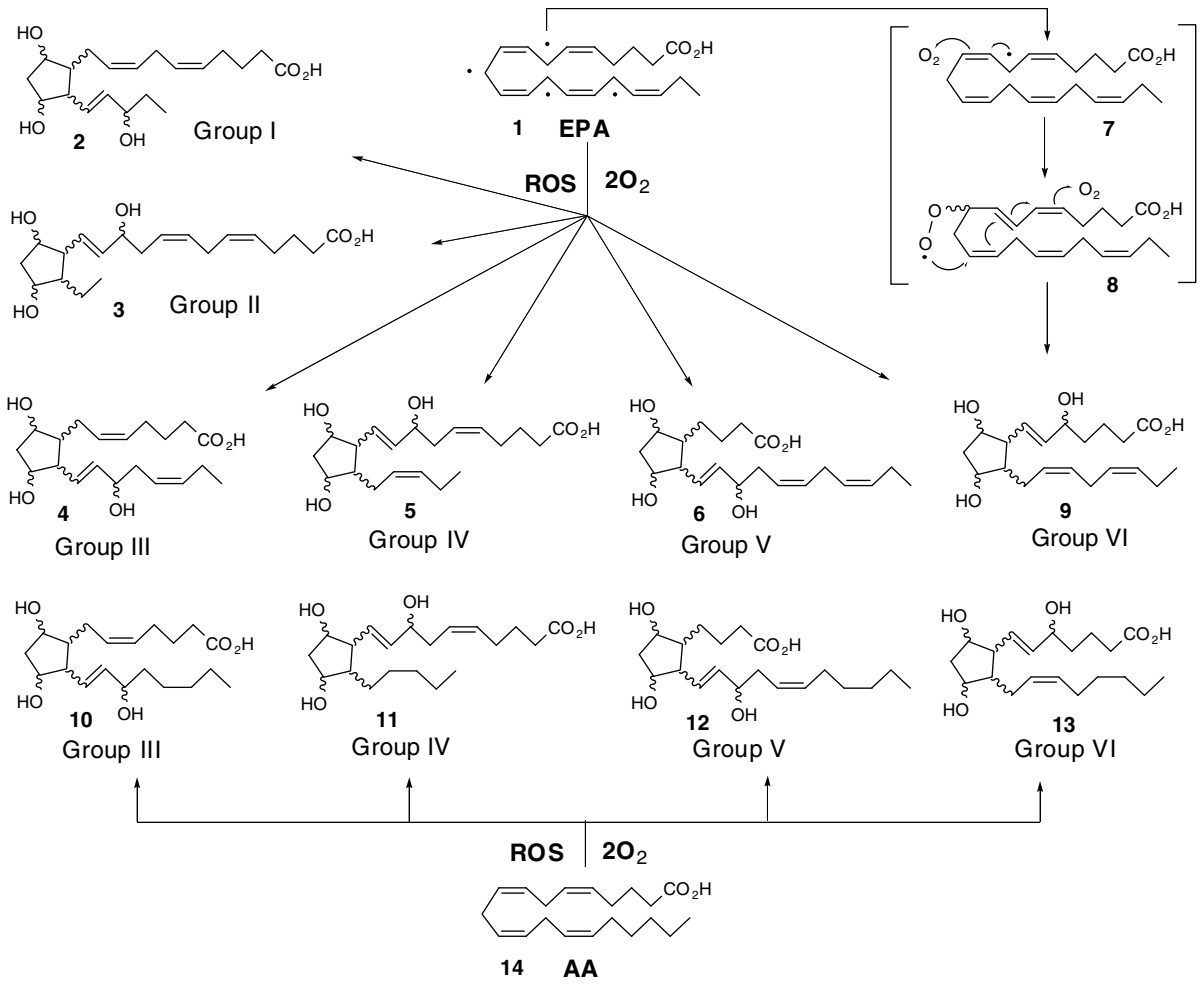
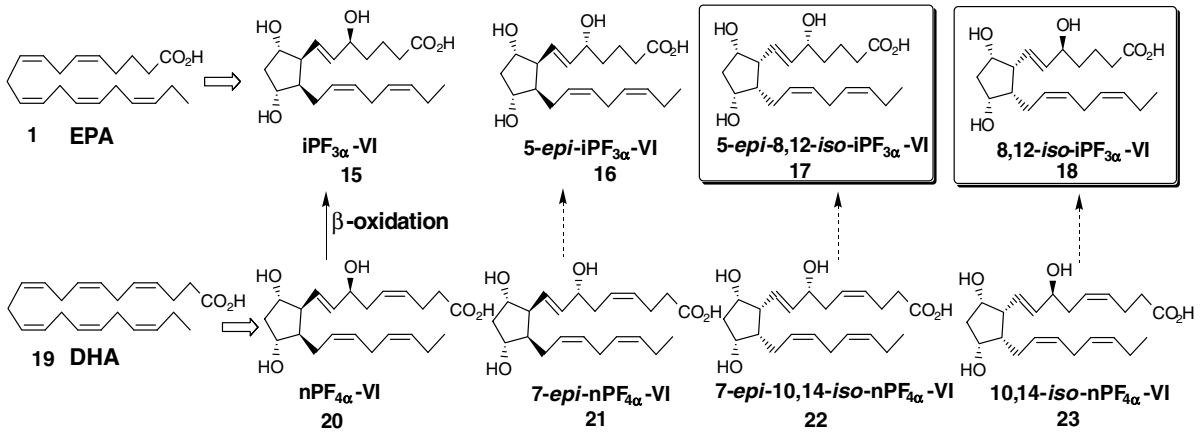


Scheme 1. Isoprostanes derived from AA and EPA.



Scheme 2. Isoprostanes derived from Neuroprostanes by β-oxidation.

were the case we would stand a much higher chance of finding increased amounts of intact Group VI iPs in urine. As a result, we have discovered that AA-derived Group VI iPs **13**, Scheme 1, were the most abundant AA-derived iPs in urine.[13]

In addition, we have carried out very recently a detailed study[8] in which we have been able to demonstrate that **15** is resistant to β-oxidation and that **20**, a member of the neuroprostane (nP) family derived from DHA, is metabolized to **15** (Scheme 2 and Fig. 1 panel A). This also explains why attempts to detect intact neuroprostanes in urine have been unsuccessful.[14]

Having selected Group VI **9** as a higher-probability target for the non-invasive discovery of EPA-derived iPs in urine, we still needed to reduce the number of iPs to focus upon within this group to a more manageable number. Group VI, as is the case for all other groups, is composed of 16 isomers, eight of which are enantiomers. Since our analytical tool is the mass spectrometer, which cannot

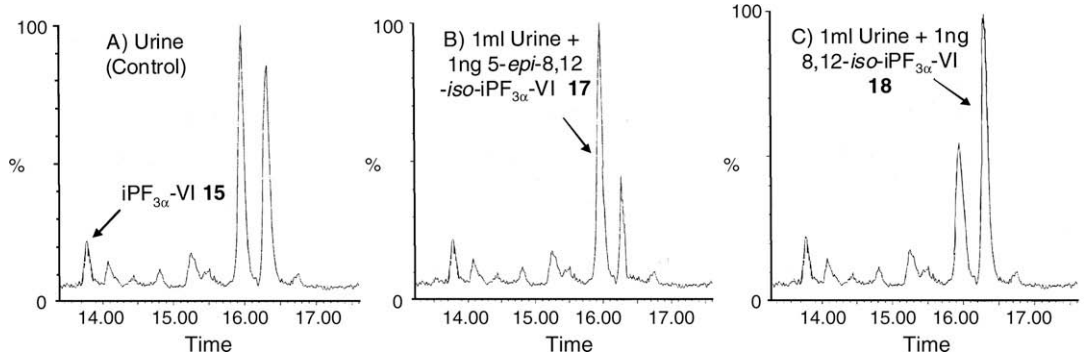
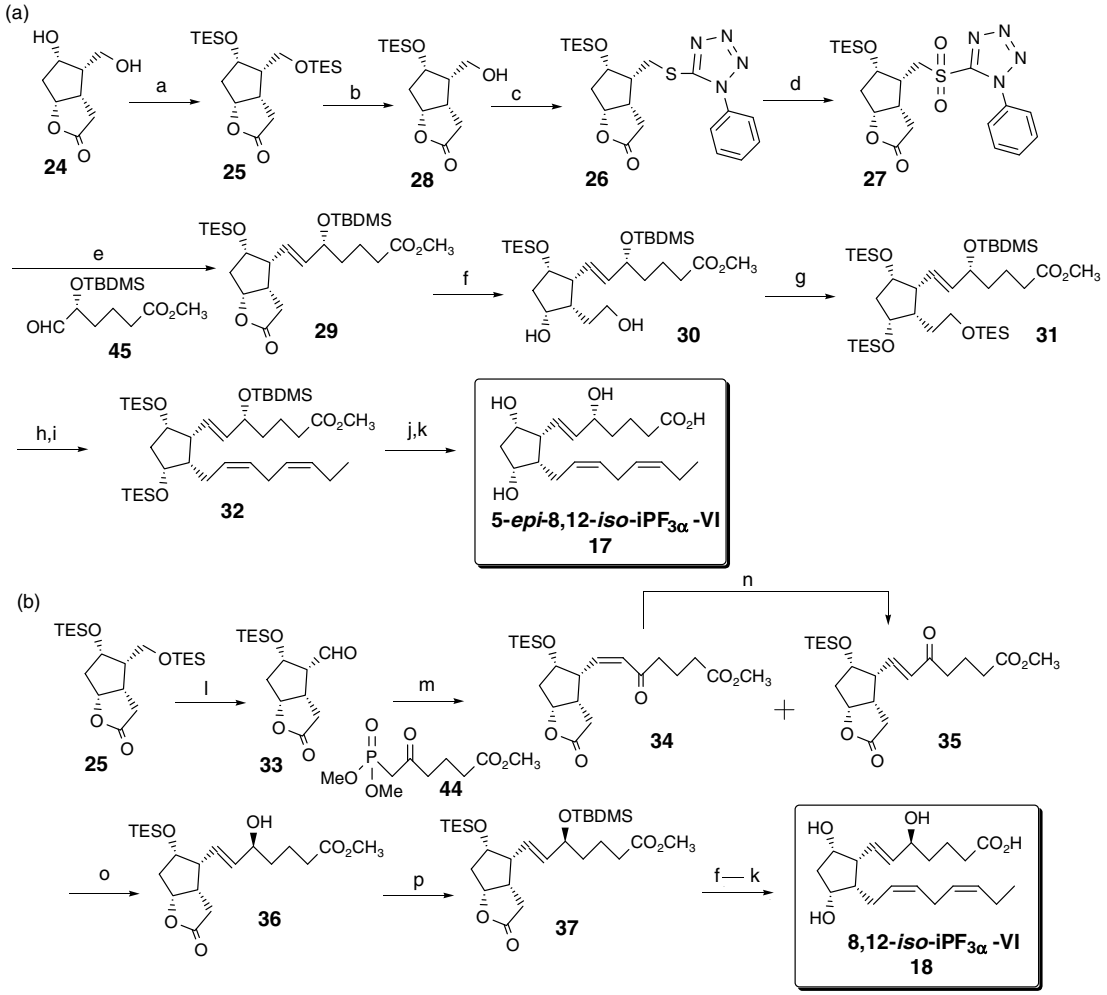


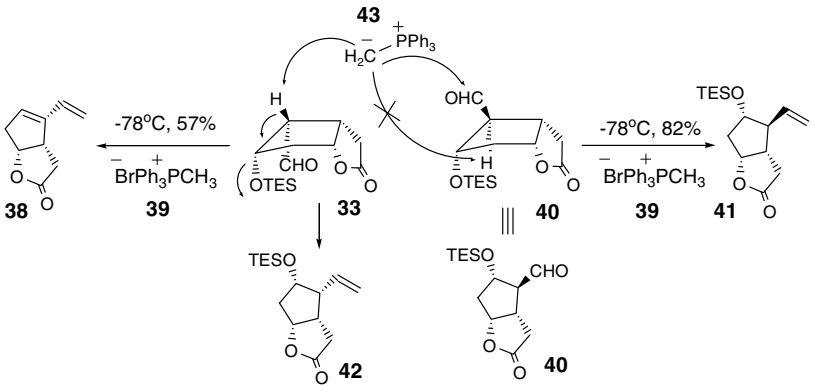
Figure 1. Identification of 5-*epi*-8,12-*iso*-iPF$_{3\alpha}$-VI **17** and 8,12-*iso*-iPF$_{3\alpha}$-VI **18** in human urine using LC/MS/MS LC/MS/MS conditions: isoprostanes were evaluated by liquid chromatography/tandem mass spectrometry (LC/MS/MS), using reverse phase chromatography, negative ion electrospray introduction and selected reaction monitoring (SRM) techniques. A 200 × 2.1 mm Hypersil Gold C18 1.9 μm particle size UHPLC column was used. The mobile phase was generated from (A), HPLC-grade water and (B) 5% methanol/95% acetonitrile, both containing 0.005% acetic acid and adjusted to pH 5.7 with ammonium hydroxide. The flow rate was 350 μL/min using a linear gradient from 20% B to 38% B in 20 min. The transitions monitored were *m/z* 351 and *m/z* 115 for both 5-*epi*-8,12-*iso*-iPF$_{3\alpha}$-VI **17** and 8,12-*iso*-iPF$_{3\alpha}$-VI **18**.

distinguish between enantiomers, it is safe to ignore these compounds for the present application. Of the eight remaining isomers, four have the side chains *trans* to each other. Since radical cyclization precedents indicate that *cis* arrangements are largely preferred,[15,16] we decided to ignore the *trans* isomers and focus on the four *cis* isomers, **15**, **16**, **17**, and **18**.

Synthesis: Scheme 3 describes the stereospecific synthesis of 5-*epi*-8,12-*iso*-iPF$_{3\alpha}$-VI **17** and 8,12-*iso*-iPF$_{3\alpha}$-VI **18**. Scheme 3(a)



Scheme 3. Stereospecific synthesis of 5-*epi*-8,12-*iso*-iPF$_{3\alpha}$-VI **17** and 8,12-*iso*-iPF$_{3\alpha}$-VI **18**. Reagents and conditions: (a) TESCl, Et_3N, DMAP, CH_2Cl_2, rt, 20 h, 98%; (b) Wilkinson's catalyst, catechol borane, THF, 0 °C–rt, overnight, 91%; (c) PT-SH, Ph_3P, DIAD, THF, 0 °C–rt, 4.5 h, 97%; (d) m-CPBA, $NaHCO_3$, CH_2Cl_2, rt, overnight, 96%; (e) KHMDS, **45**,[21] DME, −60 °C–rt, 8 h, 52% (*E*: *Z* = 63: 37); (f) $NaBH_4$, diethyl ether/MeOH (2:1), rt, 35 min, 70%; (g) TESCl, Et_3N, DMAP, CH_2Cl_2, 20 h, 95%; (h) DMSO, $(COCl)_2$, Et_3N, CH_2Cl_2, −70 to −20 °C, 1.5 h; (i) LiHMDS, THF, −78 °C to rt, $I^-Ph_3P^+(CH_2)_2CH{=}CHCH_2CH_3$, 2 h (two steps 73%); (j) TBAF, THF, rt, overnight; (k) LiOH, iPrOH/H_2O (1:1), rt, 4 h (two steps 93%); (l) DMSO, $(COCl)_2$, Et_3N, CH_2Cl_2, −70 to −20 °C, 1.5 h; (m) LiHMDS, **44**, THF, −78 °C to rt, 2 h (two steps **34**, 8.4%, **35**, 73%); (n) I_2, CH_2Cl_2, rt, 15 h, 87%; (o) EtOH, $LiAlH_4$, (*S*)-Binal-H, THF, −100 °C, 45 min, 92%; (p) TBDMSCl, Et_3N, DMAP, CH_2Cl_2, rt, overnight, 85%.



Scheme 4. Elimination of TES-OH during Wittig reaction.

shows the first total synthesis of iP **17**. The starting dihydroxy lactone **24** has been described by us previously.[4] The key feature in this approach is the Kocienski-modified Julia olefination[17] reaction between **27** and **45**. This guarantees the *R*-stereochemistry of the OTBDMS at position C-5 in **29**. However, as is usually the case in this coupling, a mixture of *cis* and *trans* (2:1) is formed which is separated by silica column chromatography and readily identified by ^{1}H NMR (*trans* and *cis* coupling of the olefins). The selective conversion of *bis*-TES **25** to the mono-TES **28** was performed in excellent yield using the Rh-catalyzed catechol borane procedure we reported recently.[18] Other methods tried were not as selective. The reaction of DIBAL on **29** produces a mixture of the lactol and the diol. We elected instead to convert the lactone to the diol **30** and proceed via the *tris*-TES **31**. Some reduction of the ester function also occurs during the reduction step and is separated by silica gel column chromatography.[19]

We have previously reported on a stereospecific synthesis of **18**.[19] The preparation of **18** as described in Scheme 3(b) is shorter and may be better suited for the preparation of some analogs and derivatives. To obtain the aldehyde **33** we used a very convenient one-step procedure[20] which avoids the need for protection/deprotection. The *S*-Binal reduction of **35** to **36** proceeded with high e.e. as judged by the LC/MS analysis of the final compound **18** (not shown).

The reactivity of aldehyde **33** merits comment. The Wittig reaction with the stabilized ketophosphonate **44**[22] proceeded uneventfully in high yield, as shown in Scheme 3. Attempts to perform a similar reaction with the more nucleophilic and basic **43** resulted mostly in the elimination of TES-OH to afford **38** (Scheme 4). Variable amounts of the target compound **42** are produced. As can be seen in the three-dimensional structure **33**, the hydrogen α to the aldehyde is exposed with little hindrance, resulting in a preferential attack of the ylide **43** on this hydrogen instead of the desired attack on the aldehyde. To prove the point we prepared **40** from commercial *ent*-Corey lactone. In this case the hydrogen is hindered and no elimination product is formed and a high yield of the olefin **41** obtained.

Discovery of 8,12-iso-iPF$_{3\alpha}$-VI and 5-epi-8,12-iso-iPF$_{3\alpha}$-VI in human urine: We have used the two synthetic probes **17** and **18** to identify these iPs in urine. It is interesting to note that the two peaks that we identified as **17** and **18** are by far the most prominent peaks in the mass chromatogram representing Group VI iPs (Fig. 1A). This is not unlike the case we encountered previously in the 14,15-dihydro series of AA-derived iPs, in which the two all-*syn* iPs in Group VI are the most prevalent.[13,23] Panels B and C of Figure 1 represent urine supplemented with 1 ng of either **17** or **18**, respectively. Various co-injections with 1–5 ng produced similar results (not shown).

A final comment on the origin of these two peaks: we have previously shown that **20** (Scheme 2) is metabolized to **15**, suggesting that urinary **15** is derived from a combination of direct formation from EPA, as shown in Scheme 1, and β-oxidation of the DHA-derived nPs **20**.[8] It is tempting to assume that in this case, too, urinary **17**[24] and **18**[24] may have arisen as a result of these two pathways, and may be good in vivo indicators of peroxidation of both EPA- and DHA-containing lipids.

Acknowledgments

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24. Spectral data of **5-*epi*-8,12-*iso*-iPF$_{3\alpha}$-VI (17)** ^{1}H NMR (Methanol-d$_4$, 400 MHz) δ 5.712(1H, dd, J = 15.3, 10.6), 5.439-5.320 (2H, m), 5.281-5.155 (3H, m), 4.094–3.925 (3H, m), 2.768–2.641 (2H, m), 2.589–2.482 (1H, m), 2.391–2.285 (1H, m), 2.268–1.921 (6H, m), 1.780–1.681 (1H, m), 1.635–1.371 (5H, m), 0.866 (3H, t, J = 7.5); ^{13}C NMR (methanol-d_4, 100 MHz) δ 177.59, 137.48, 132.59, 130.12, 129.58, 129.40, 128.66, 74.88, 73.08, 72.86, 52.03, 48.65, 43.53, 37.86, 35.30, 26.65, 25.11, 22.40, 21.48, 14.67; HRMS m/z calcd for $C_{20}H_{31}O_4^+$ 335.2222 found 335.2221.**8,12-*iso*-iPF$_{3\alpha}$-VI (18)** ^{1}H NMR (methanol-d_4, 400 MHz) δ 5.771 (1H, dd, J = 15.3, 10.7), 5.380–5.149 (5H, m), 4.098–3.924 (3H, m), 2.771–2.643 (2H, m), 2.581–2.495 (1H, m), 2.393–2.291 (1H, m), 2.249–1.942 (6H, m), 1.779–1.661 (1H, m), 1.623–1.369 (5H, m), 0.868 (3H, t, J = 7.5); ^{13}C NMR (Methanol-d_4, 100 MHz) δ 177.58, 137.48, 132.60, 130.98, 129.98, 129.44, 128.58, 74.83, 73.82, 72.79, 51.95, 48.49, 43.56, 37.61, 34.95, 26.66, 25.12, 22.29, 21.50, 14.68; HRMS m/z calcd for $C_{20}H_{31}O_4^+$ 335.2222 found 335.2221.