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# First Total Syntheses of Novel Non-Enzymatic Polyunsaturated Fatty Acid Metabolites and Their Identification in Edible Oils

Tereza Pavlíčková,<sup>[a, b, c]</sup> Valérie Bultel-Poncé,<sup>[a]</sup> Alexandre Guy,<sup>[a]</sup> Amandine Rocher,<sup>[a]</sup> Guillaume Reversat,<sup>[a]</sup> Claire Vigor,<sup>[a]</sup> Thierry Durand,<sup>[a]</sup> Jean-Marie Galano,<sup>\*,[a]</sup> Ullrich Jahn,<sup>\*,[b]</sup> and Camille Oger<sup>\*,[a]</sup>

**Abstract:** Oxidative stress (OS) is an in vivo process leading to free radical overproduction, which triggers polyunsaturated fatty acid (PUFA) peroxidation resulting in the formation of racemic non-enzymatic oxygenated metabolites. As potential biomarkers of OS, their in vivo quantification is of great interest. However, since a large number of isomeric metabolites is formed in parallel, their quantification remains difficult without primary standards. Three new PUFA-metabolites, namely 18-F<sub>3t</sub>-isoprostane (IsoP) from eicosapentaenoic acid (EPA), 20-F<sub>4t</sub>-neuroprostane (NeuroP) from docosahex-

aeonic acid (DHA) and 20-F<sub>3t</sub>-NeuroP from docosapentaenoic acid (DPA<sub>n-3</sub>) were synthesized by two complementary synthetic strategies. The first one relied on a racemic approach to 18(*RS*)-18-F<sub>3t</sub>-IsoP using an oxidative radical anion cyclization as a key step, whereas the second used an enzymatic deracemization of a bicyclo[3.3.0]octene intermediate obtained from cyclooctadiene to pursue an asymmetric synthesis. The synthesized metabolites were applied in targeted lipidomics to prove lipid peroxidation in edible oils of commercial nutraceuticals.

## Introduction

The discovery of non-enzymatic peroxidation of arachidonic acid (AA) by Morrow et al. thirty years ago opened a new research field in eicosanoids.<sup>[1]</sup> Unlike the enzymatic biosynthesis of prostaglandins (PGs), the non-enzymatic AA metabolism is driven by reactive oxygen species (ROS), which are over-produced under oxidative stress conditions and can react with AA glycerophospholipids by free radical reaction cascades (similar to PG biosynthesis from free AA) to generate end-metabolism oxylipins like racemic isoprostanes (IsoPs). Nowadays IsoPs are

widely accepted as biomarkers of oxidative stress and participate in several physiological processes. IsoPs have been shown to act as lipid mediators in vasoconstriction and platelet aggregation, and may participate in intracellular signaling through activation of prostanoid receptors.<sup>[2]</sup> Similar radical oxidation/cyclization reactions take place with all other PUFAs such as DHA (docosahexaenoic acid, C22:6 n-3), EPA (eicosapentaenoic acid, C20:5 n-3) or DPA<sub>n-3</sub> (docosapentaenoic acid, C22:5 n-3) from the omega-3 family or AdA (adrenic acid, C22:4 n-6) as an omega-6 PUFA.<sup>[3]</sup> A large number of cyclic metabolites are formed during lipid peroxidation, which necessitates a classification and specific nomenclature for each family of oxylipins.<sup>[4]</sup> Thus, cyclic compounds resulting from peroxidation of fatty acids with 18 carbon atoms are called phytoprostanes (PhytoPs), those with 20 carbon atoms IsoPs, and those with 22 carbon atoms neuroprostanes (NeuroPs). They also differ from each other by the number of double bonds. Importantly, each of these multiple individual compounds may have its own biological role(s). We recently demonstrated that a particular isomer of NeuroP from DHA is a powerful anti-arrhythmic compound,<sup>[5]</sup> an IsoP from EPA is neuroprotective in bovine retina,<sup>[6]</sup> whereas a PhytoP from  $\alpha$ -linolenic acid (ALA) protects immature neurons from oxidant injury and promotes differentiation of oligodendrocyte progenitors.<sup>[7]</sup>

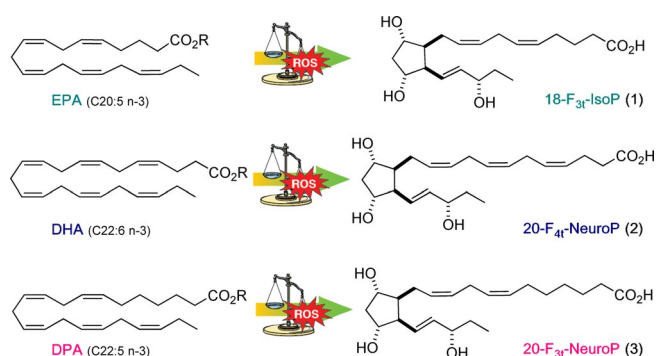
Our two groups have been working on the synthesis of these non-enzymatic PUFA metabolites for many years and developed diverse individual synthetic strategies but we decided to work together to target new metabolite families. Racemic IsoPs are accessible by Jahn's oxidative cyclization/ $\alpha$ -chain attachment strategy (Approach I).<sup>[8-10]</sup> Since potential specific biological activity is likely inherent to individual enantiomers,

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asymmetric approach based on enzymatic deracemization of bicyclo[3.3.0]octenes developed in the Durand group will serve to access them for in depth study (Approach II).<sup>[11,12]</sup> Merging the two strategies can be expected to accelerate specification of individual metabolites significantly. Here, we focus our efforts on the synthesis of C20 and C22 metabolites having the 3-hydroxypentenyl  $\omega$ -chain as common feature. This includes providing access to one of the major cyclic EPA metabolites, namely 18-F<sub>3t</sub>-IsoP (1) to prove its in vivo formation and to further use it as primary standard in targeted lipidomics. Our strategy allows to attach in parallel various  $\alpha$ -chain modules leading to a unified approach to NeuroP derived from DHA (2) and DPA<sub>n-3</sub> (3) using the same precursor (Scheme 1).



Scheme 1. Formation of the target families of oxylipins.

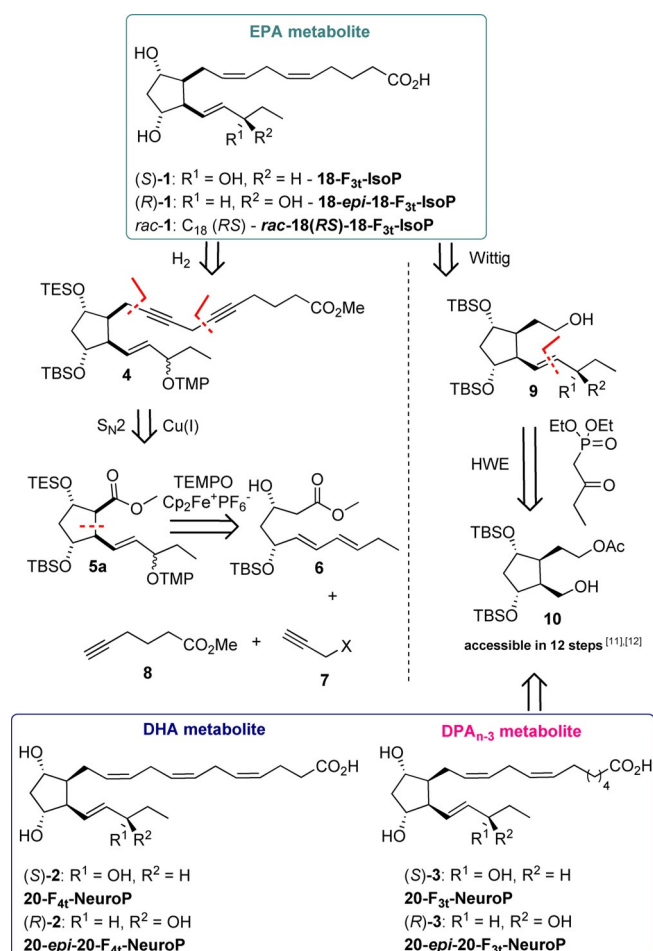
Here, we report the first total syntheses of racemic 18(*RS*)-18-F<sub>3t</sub>-IsoP (*rac*-1), 18-F<sub>3t</sub>-IsoP (*S*-1) and 18-*epi*-18-F<sub>3t</sub>-IsoP (*R*-1), 20-F<sub>4t</sub>-NeuroP and 20-*epi*-20-F<sub>4t</sub>-NeuroP (*S*-2) and (*R*-2) as well as 20-F<sub>3t</sub>-NeuroP and 20-*epi*-20-F<sub>3t</sub>-NeuroP (*S*-3) and (*R*-3) epimers, respectively. We apply the synthesized cyclic EPA and DHA metabolites as analytical standards to identify the formation of lipid peroxidation metabolites in two encapsulated edible oil nutraceuticals.

## Results and Discussion

### Retrosynthetic analysis

The retrosynthetic analysis of racemic 18(*RS*)-18-F<sub>3t</sub>-IsoP (*rac*-1, approach I) leads to protected skipped diyne **4**, which can be further simplified by twofold C(sp<sup>3</sup>)-C(sp) disconnection to oxygenated cyclopentanecarboxylate **5a**, propargylic unit **7** and methyl hex-5-ynoate **8** (Scheme 2). The cyclic intermediate **5a** can be further disconnected to protected 3,5-dihydroxy ester **6**.<sup>[10]</sup>

The asymmetric approach II envisioned disconnection of enantiomerically enriched **1**, **2** and **3** at the (*Z*)-double bond closest to the ring leading to common enantiomerically enriched 2-hydroxyethylcyclopentane **9** with the  $\omega$ -chain in place. Further disconnection of the remaining double bond leads to cyclopentane **10**, which can be accessed on multi-gram scale by enzymatic deracemization.<sup>[12]</sup>



Scheme 2. Retrosynthetic analysis. TMP = 2,2,6,6-tetramethylpiperidin-1-yl.

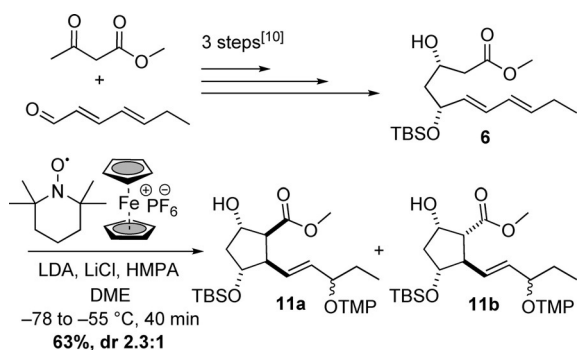
### Approach I: Total synthesis of *rac*-18(*RS*)-18-F<sub>3t</sub>-IsoP (*rac*-1)

The synthesis commenced with cyclization precursor **6** readily available in three steps in high yield,<sup>[10]</sup> which was deprotonated by excess LDA in the presence of 8.5 equiv LiCl, followed by oxidative cyclization of the resulting alkoxide enolate trig-

Camille Oger was born in Saint-Gaudens (France). She obtained her PhD from Montpellier 2 University in the field of total synthesis in 2010 and she did postdoctoral work with Pr. Ilan Marek at the Technion- Israel Institute of Technology, working on a new access to polypropionate units. She was promoted assistant professor at Montpellier 1 University in 2011, and obtained her habilitation in 2016 based on her research work on the development of new strategies toward the synthesis of bioactive lipids. In 2017, she received Jean Normant award from the Organic Division of the French Chemical Society. Her research field is at the interface of chemistry and biology, with the total synthesis of lipid metabolites, the quantification of such compounds in biological fluids and the research of potent biological properties.

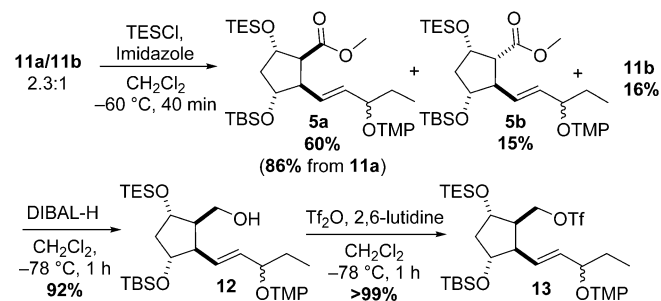


gered by ferrocenium hexafluorophosphate and oxygenation by stable free radical TEMPO (Scheme 3). Hydroxycyclopentane carboxylates **11a** and **11b** were isolated in 63% yield and slightly improved 2.3:1 diastereoselectivity compared to our previous cyclization conditions.<sup>[10]</sup>



**Scheme 3.** Synthesis of cyclopentanols **11a/11b**.

Treating the inseparable diastereomeric mixture of **11a** and **11b** with a strictly equimolar amount of TESCl resulted in the formation of a chromatographically easily separable mixture of 60% of desired *cis*-isomer **5a** (86% with respect to **11a**), 15% of **5b** and 16% of recovered **11b** (Scheme 4). Isolated ester **5a** was reduced by DIBAL-H in high yield and cleanly transformed to the corresponding triflate **13**, thus providing the electrophilic alkylation partner.



**Scheme 4.** Preparation of triflate **13** from esters **11a/11b**.

The  $\alpha$ -chain was connected in a two-step procedure. At first a propargylic unit was installed as a double bond surrogate. Several propargylic precursors were tested (Table 1). PMB-protected propargylic alcohol **7a** was a suitable difunctional reactant. Its deprotonation by *n*BuLi smoothly proceeded and gave propargylic cyclopentane **14a** in good yield (entry 1), however, the subsequent deprotection of the PMB group (not shown) was not successful. Propargylic alcohol-derived dianion should also give the desired outcome after O- and C-deprotonation, but would still require further transformation of the hydroxy group.

A more direct approach consists of alkylation of propargylic halides, but this requires carefully controlled conditions. In the

**Table 1.** Introduction of propargylic unit **7** by acetylide alkylation.

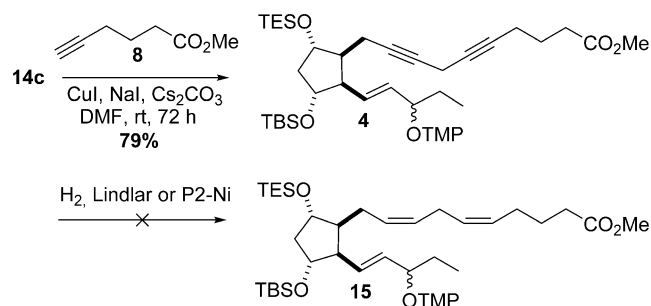
Entry	X	Base	Et <sub>2</sub> O/HMPA	Conditions	<b>14</b> (%)
1	OPMB	<i>n</i> BuLi	4:1 <sup>[a]</sup>	-78 °C to -10 °C, 1.5 h	64
2	Br	LDA	10:1	-90 °C to 0 °C, 1 h	0
3	Cl	<i>n</i> BuLi	10:1	-85 °C to rt., 16 h	32
4	Cl	<i>n</i> BuLi	10:1	-85 °C to 0 °C, 2.5 h	65
5	Cl	LDA	10:1	-85 °C to -40 °C, 2 h	77
6	Cl	LDA	20:1	-85 °C to -40 °C, 2 h	32
7	Cl	LDA	10:1	-110 °C to -50 °C, 1 h	81

[a] In THF instead of Et<sub>2</sub>O.

event, when propargylic bromide **7b** was subjected to LDA, the reaction gave a complex mixture of products that further decomposed during column chromatography and (bromobutynyl)cyclopentane **14b** could not be isolated (entry 2). In contrast, the use of propargylic chloride **7c** and slow warming was successful albeit in low yield (entry 3). Further optimization revealed that shortening the reaction time to 2 h, while warming to 0 °C significantly improved the yield of (chlorobutynyl)cyclopentane **14c** to an acceptable level (entry 4, 65% yield). Switching back to LDA and raising the temperature to -40 °C over two hours led to further improvement (entry 5, 77% yield), however decreasing the amount of HMPA resulted in a sharp drop of the yield (entry 6, 32% yield). Finally, we were pleased to find the optimal conditions, which consisted of deprotonating **7c** at -110 °C and raising the reaction temperature to maximally -50 °C over an hour, providing **14c** in 81% yield (entry 7). This transformation might be very useful for constructing other skipped polyene systems.

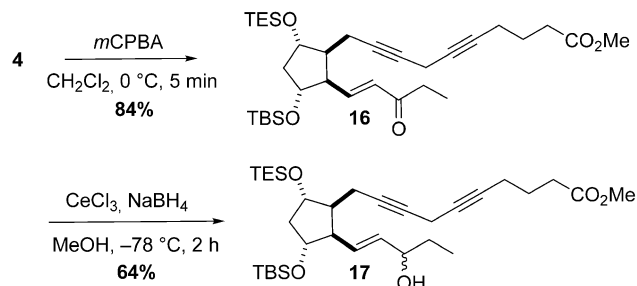
Subsequent Cu<sup>I</sup> mediated C-C coupling of 4-cyclopentyl-2-butynyl chloride **14c** with 5-hexynoate **8**<sup>[13]</sup> proceeded with good yield and allowed assembly of the full carbon skeleton of ester **4** (Scheme 5). Partial hydrogenation of this fully protected compound was attempted, however, numerous applied conditions gave mixtures of partially, fully and overhydrogenated compounds (not shown).

Our experience suggested that a free hydroxy group might augment the selectivity thanks to better coordination of the



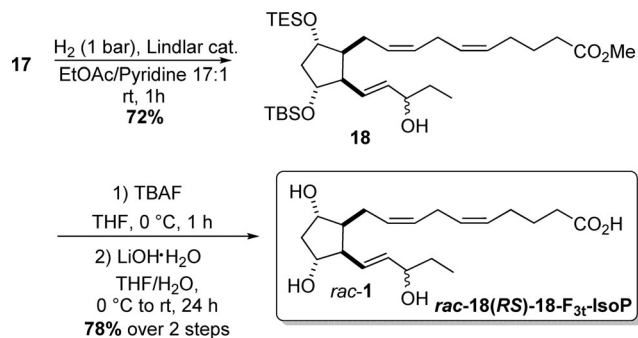
**Scheme 5.** Cu<sup>I</sup>-Mediated coupling and failed hydrogenation of diyne **4**.

substrate to the catalyst surface. Therefore, the tetramethylpiperidyl (TMP) group in diyne ester **4** was cleaved under oxidative conditions affording enone **16** in very good yield. It should be noted that despite a potential sensitivity of the skipped diyne to the oxidative conditions, a short reaction time guaranteed a clean reaction. Subsequent reduction of the carbonyl group in **16** under Luche conditions furnished allylic hydroxy ester **17** as a 1.2:1 mixture of C-18 epimers in 64% yield (Scheme 6).



Scheme 6. Oxidative TEMPO deprotection and Luche reduction.

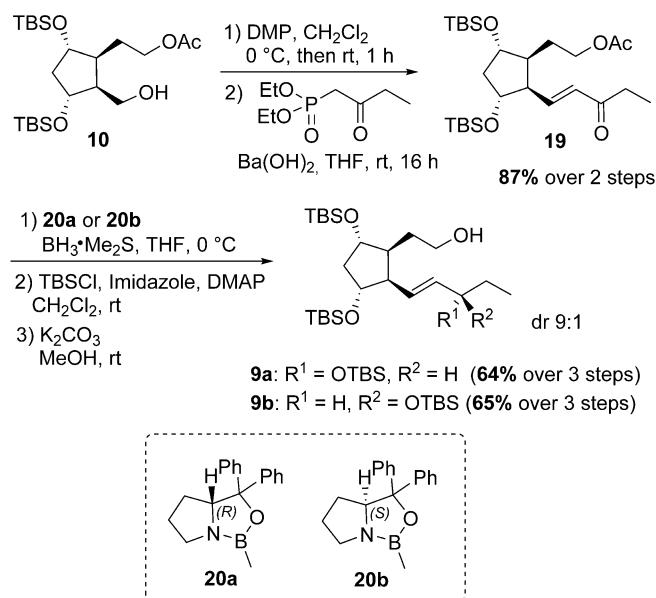
Hydroxydiynoate **17** was subjected to hydrogenation under typical Lindlar conditions (EtOAc/pyridine),<sup>[8]</sup> which gratifyingly gave skipped diene **18** in 72% yield (Scheme 7). Finally, the remaining silyl groups were cleaved with tetrabutylammonium fluoride (TBAF) and final saponification with LiOH-H<sub>2</sub>O provided racemic 18(*RS*)-18-F<sub>3t</sub>-IsoP (*rac*-**1**) as a partially separable mixture of C-18 epimers. Thus, the total synthesis of *rac*-**1** was accomplished in 7% yield over 11 steps from known monoprotected 3,5-dihydroxy ester **6** (5% over 14 steps in total).



Scheme 7. Hydrogenation of **17** and final deprotection.

### Approach II: Asymmetric total syntheses of 18-F<sub>3t</sub>-IsoP, 20-F<sub>4t</sub>-NeuroP, and 20-F<sub>3t</sub>-NeuroP (1–3)

The asymmetric syntheses of 18-F<sub>3t</sub>-IsoP, 20-F<sub>4t</sub>-NeuroP and 20-F<sub>3t</sub>-NeuroP **1–3** began with the preparation of monoacetylated cyclopentane **10** on gram scale in 10% overall yield over 12 steps.<sup>[11,12]</sup> As previously described, enzymatic deracemization using *Amano AK* lipase permitted access to properly functionalized enantioenriched compound **10**.<sup>[11]</sup> The  $\omega$ -chain was subsequently introduced over five steps (Scheme 8). Enone **19**



Scheme 8. Preparation of common intermediates **9a** and **9b**.

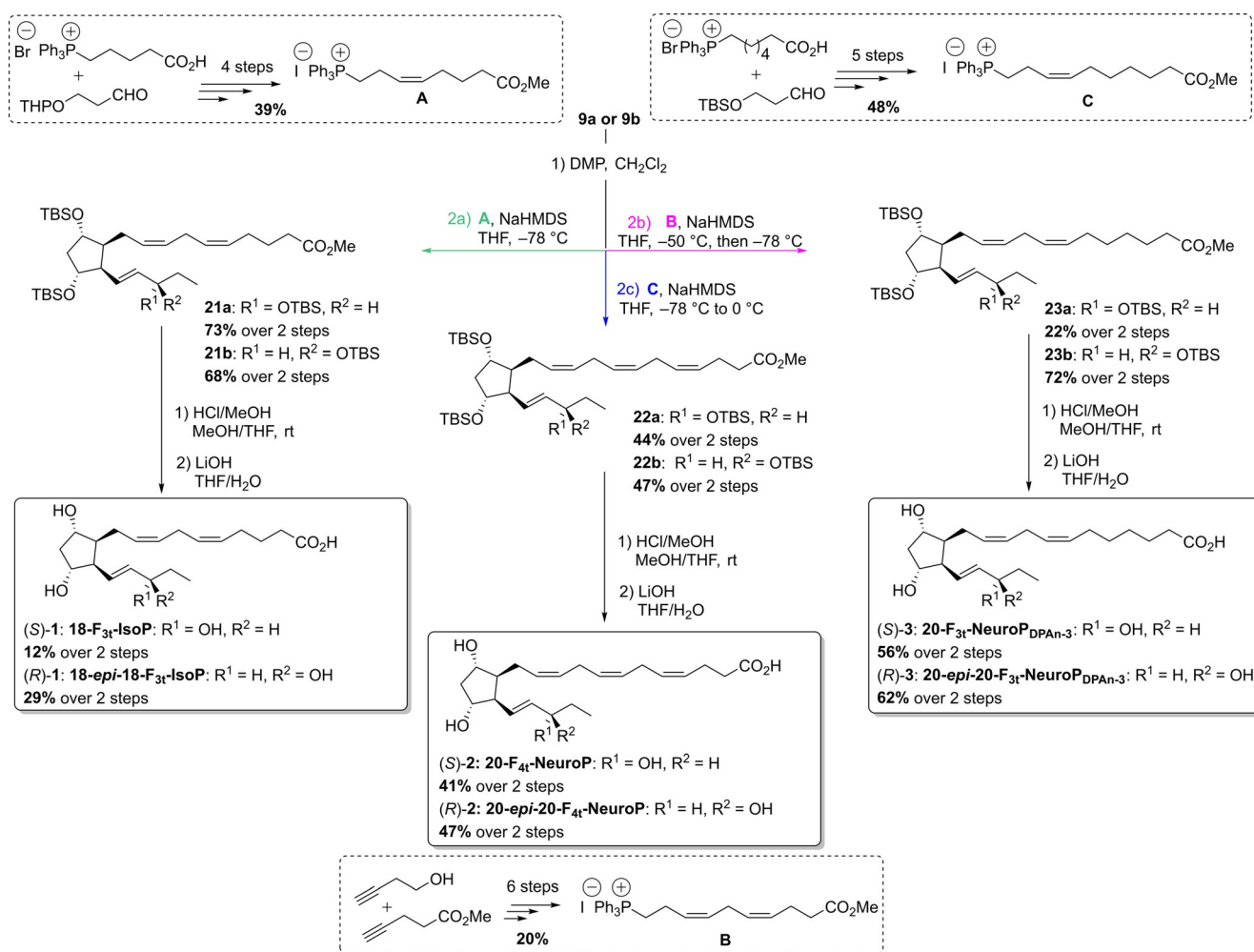
was cleanly prepared in 87% yield by a two-step sequence consisting of Dess–Martin oxidation of the primary alcohol followed by a Horner–Wadsworth–Emmons (HWE) reaction with dimethyl 2-oxobutylphosphonate using Ba(OH)<sub>2</sub> as base under mild conditions to avoid epimerization.<sup>[15]</sup>

The keto group in **19** was subsequently enantioselectively reduced using a Corey–Bakshi–Shibata (CBS) reduction with (*R*)-(+)- or (*S*)-(–)-2-methyl-1,3,2-oxazaborolidines **20a** or **20b**. Both, the corresponding (*S*)- and (*R*)-allylic alcohols were obtained in good yields, each in a 9:1 diastereoisomeric ratio (*dr*). TBS-protection of the newly formed hydroxy group and acetate deprotection completed the synthesis of common intermediates (*S*)-**9a** and (*R*)-**9b** in 64% and 65% yield, respectively, over three steps.

With central precursors **9** containing the cyclopentane ring and the  $\omega$ -chain at hand, the syntheses of EPA, DHA and DPA<sub>n-3</sub> metabolites were pursued, all three following the same synthetic route (Scheme 9). 2-Cyclopentylethanols **9a** and **9b** were first oxidized to the corresponding aldehydes using the Dess–Martin periodinane and subsequently subjected to Wittig reactions to install the  $\alpha$ -side chain.

The desired phosphonium salts **A**, **B** and **C**, were synthesized in four to six steps, respectively (see the Supporting Information for the synthesis). The corresponding ylides were generated by deprotonation with NaHMDS at low temperature (–50 °C or –78 °C) before reaction with the aldehydes derived from **9a** or **9b**. Fully protected derivatives of 18-F<sub>3t</sub>-IsoP, 20-F<sub>4t</sub>-NeuroP and 20-F<sub>3t</sub>-NeuroP **21a,b**–**23a,b** were obtained in moderate to good 22 to 73% unoptimized yields. The yields of the Wittig reactions were dependent on the nature of the phosphonium salts (see Supporting Information).

With the fully assembled carbon skeleton at hand, final deprotection steps were carried out on compounds **21–23**. First, the three silyl groups were hydrolyzed with 0.5 M HCl in MeOH. To our delight, we were able to separate the approxi-



**Scheme 9.** Asymmetric synthesis of 18-F<sub>3t</sub>-IsoP, 20-F<sub>4t</sub>-NeuroP and 20-F<sub>3t</sub>-NeuroP and their epimers at the hydroxy group in the ω-chain.

mately 10% of undesired epimers carried through from the CBS reductions at this stage by silica gel column chromatography. Partial hydrolysis of the methyl ester to target 20-F<sub>3t</sub>-NeuroP **3** was also observed under these conditions. Finally, saponification of the methyl esters using LiOH·H<sub>2</sub>O provided the individual 18- and 20-epimers of 18-F<sub>3t</sub>-IsoP **1**, 20-F<sub>4t</sub>-NeuroP **2** and 20-F<sub>3t</sub>-NeuroP **3** in 12–62% yield over the two steps. Thus, we obtained compounds (*R*)-**1** in 11% yield, (*S*)-**1** in 5% yield, (*R*)-**2** in 13% yield, (*S*)-**2** in 10% yield, (*R*)-**3** in 25% yield, (*S*)-**3** in 7% yield over nine steps from monoacetylated cyclopentane **10**.

### Quantification of compounds in edible oils

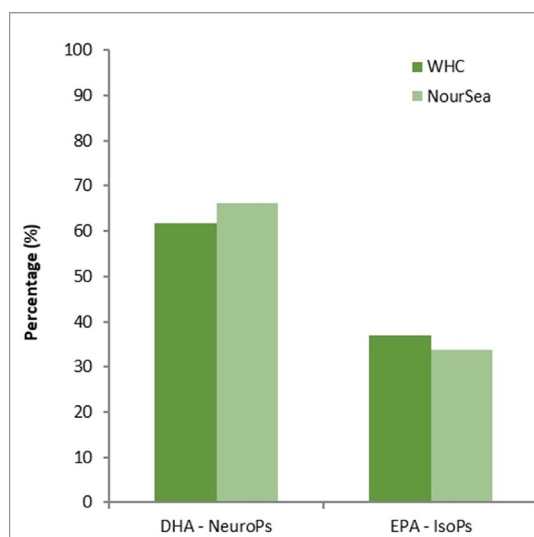
Almost fifty years ago, Bang and Dyerberg reported low incident rates of cardiovascular diseases in Greenland Inuits who consumed mainly omega-3 fatty acids by intake of seal meat.<sup>[16]</sup> Since this study and many more, the food supplement industry has developed, among others, capsules rich in omega-3 PUFAs, containing mainly DHA and EPA, extracted from fat fishes or algae.<sup>[17]</sup> PUFAs have indeed essential physiological roles but are also prone to peroxidation leading to oxy-

genated metabolites. Moreover, these PUFA metabolites have found a strong biological interest on their own.<sup>[2]</sup> As one example, our group recently described the anti-arrhythmic properties of a NeuroP and has shown that this DHA metabolite has a higher activity than DHA itself.<sup>[5]</sup> Lipid peroxidation of food supplements thus represents a phenomenon not to be neglected. There are several assays to quantify lipid peroxidation reported in the literature including 15-F<sub>2t</sub>-IsoP.<sup>[18]</sup> However, this arachidonic acid metabolite is not specific to quantify the extent of peroxidation of other PUFA metabolites. Our group is dedicated to the synthesis of oxygenated PUFA metabolites to use them as biomarkers of lipid peroxidation and to highlight their individual biological properties. In this context, we recently developed a targeted lipidomic approach to quantify metabolites of all PUFAs in various matrices. In this work, we studied two commercial dietary supplements encapsulating omega-3 PUFAs stemming from different marine resources: calanus oil from *Calanus finmarchicus* crustacean (NourSea) and a fish oil mixture from sardine, anchovy, mackerel, and herring (WHC), both containing antioxidant-like compounds (astaxanthin and cholecalciferol, respectively).<sup>[19]</sup> We applied a targeted lipidomic approach using micro LC-MS/MS,<sup>[20]</sup> and quantified free metab-

olites including newly synthesized *rac*-1, (*S*)-2 and (*R*)-2, specific to DHA and EPA peroxidation.<sup>[21]</sup>

Figure 1 represents the sum of the metabolites of DHA (NeuroPs) and EPA (IsoPs) detected, which are depicted as relative percentages (the sum of NeuroPs and IsoPs being 100%). This shows a higher level of DHA metabolites compared to EPA metabolites in both oils but no significant difference between the two oils.

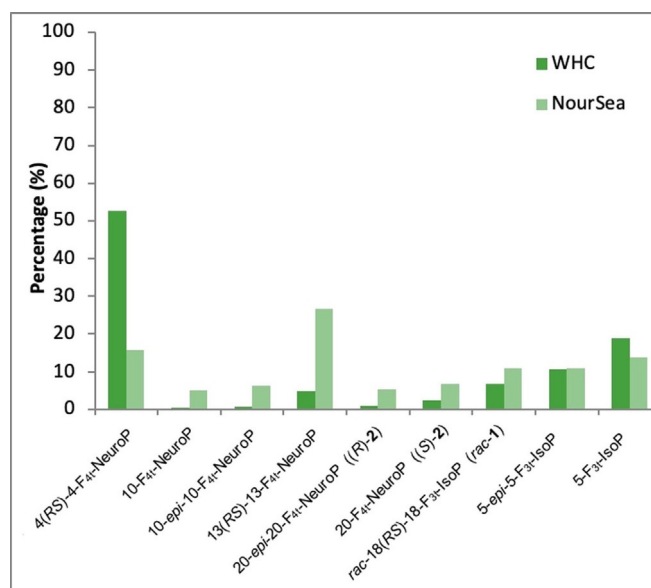
Figure 2 displays individual DHA and EPA metabolite levels as expressed by percentage of the sum of DHA and EPA metabolites, respectively.



**Figure 1.** Relative percentage of DHA and EPA derived metabolites in Nour-Sea and WHC oils.

As described in the literature, the 4- and 5-series predominate for DHA and EPA metabolites, respectively.<sup>[3a]</sup> Newly synthesized 18-*F*<sub>3t</sub>-IsoP as well as the 20-*F*<sub>4t</sub>-NeuroP were detected in sizeable quantity in both oils. The metabolite profiles vary between the two oils. A broad and relatively even distribution of all *F*<sub>4</sub>-NeuroP and *F*<sub>3</sub>-IsoP metabolites was found in calanus oil (Noursea), whereas fish oil (WHC) predominately contains 4-*F*<sub>4t</sub>-NeuroP, 5-*F*<sub>3t</sub>-IsoP and small amounts of 13-*F*<sub>4t</sub>-NeuroP and 18-*F*<sub>3t</sub>-IsoP. Both, natural oil composition and manufacturing process may play a role in this diversity. A preliminary quantification study ranges the DHA metabolites from 3 ng g<sup>-1</sup> to 230 ng g<sup>-1</sup> of oil, and EPA metabolites from 26 ng g<sup>-1</sup> to 36 ng g<sup>-1</sup> of oil.

This work is the first to report the detection and quantification of 18-*F*<sub>3t</sub>-IsoP and 20-*F*<sub>4t</sub>-NeuroP in edible oils thanks to their syntheses. More importantly, this work is the first to demonstrate the presence of several lipid peroxidation metabolites of the isoprostanoid family in food supplements. Among all the metabolites quantified in these two edible oils, we also detected 4(*RS*)-4-*F*<sub>4t</sub>-NeuroP and 5-*epi*-5-*F*<sub>3t</sub>-IsoP metabolites, which recently showed anti-arrhythmic<sup>[5]</sup> and neuroprotective<sup>[6]</sup> (in bovine retina) properties. We thus have in hands a very useful technique to highlight and quantify lipid peroxidation,



**Figure 2.** Relative percentage of DHA and EPA derived metabolites in Nour-Sea and WHC oils.

complementary to other existing methods.<sup>[18]</sup> Moreover, thanks to our database of more than fifty PUFA metabolites including PhytoPs, IsoPs and NeuroPs, our technique is specific to the particular fatty acid contained in these oils or food supplements. As of now, we focus our efforts on the detection of our synthesized metabolites in other food supplements in order to highlight lipid peroxidation of the PUFAs contained in these nutraceuticals.

## Conclusions

In summary, the first total synthesis of racemic 18(*RS*)-18-*F*<sub>3t</sub>-isoprostanone *rac*-1 was accomplished in 7% yield over 11 steps, providing a straightforward approach to this metabolite, which is suitable for determining the global configurational isomer content of the racemic analyte by LC-MS/MS studies. The cyclic core was assembled through an oxidative cyclization strategy; the complex  $\alpha$ -chain was assembled in two parts by alkylation of a propargylic chloride and Cu<sup>I</sup>-mediated coupling; final semi-hydrogenation gave the skipped diene skeleton. With this strategy, the total synthesis of 20-NeuroPs becomes feasible by using modified  $\alpha$ -chain precursors. The orthogonal protection pattern will allow a general access to IsoP and NeuroP classes with hydroxy ketone or enone ring substitution pattern.

The asymmetric total syntheses of 18-*F*<sub>3t</sub>-IsoP, 18-*epi*-18-*F*<sub>3t</sub>-IsoP, 20-*F*<sub>4t</sub>-NeuroP and 20-*epi*-20-*F*<sub>4t</sub>-NeuroP and 20-*F*<sub>3t</sub>-NeuroP and 20-*epi*-20-*F*<sub>3t</sub>-NeuroP were achieved in nine steps from monoacetate **10** and yields range from 5 to 25%. The cyclopentane ring containing four out of the five stereogenic centers was easily prepared on ten-gram scale. The  $\omega$ -chain was first installed by HWE reaction, providing a common intermediate from which the various  $\alpha$ -chains were installed by Wittig reactions with the appropriate phosphonium salts to access the desired target metabolites. Thanks to this highly divergent

approach, biological evaluation of individual IsoP and NeuroP enantiomers will be possible.

We highlighted the presence of 18-F<sub>3t</sub>-IsoP and 20-F<sub>4t</sub>-NeuroP in two encapsulated DHA/EPA food supplements. This discovery is evidence of lipid peroxidation, which might have occurred before, during or after encapsulation despite the presence of antioxidants astaxanthin or vitamin D3. More importantly, we report a targeted lipidomic method to quantify lipid peroxidation in oils, which is complementary to the known assays, and may be more specific depending on the PUFA composition of the oils to be studied. Finally, combining the results of targeted lipidomics and the access to individual PUFA metabolites by total syntheses will probably allow much more detailed biological studies of these molecules.

## Experimental Section

### Total Syntheses

**rac-18(RS)-18-F<sub>3t</sub>-Isoprostane (rac-1):** Disilylated ester **18** (3.0 mg, 6.3 μmol) was dissolved in dry THF (250 μL) under a nitrogen atmosphere, the solution was cooled to 0 °C and TBAF (13 μL, 1.0 M in THF, 13 μmol) was dropwise added. The dark yellow solution was stirred at 0 °C for 1 h when the reaction was complete as indicated by TLC. The mixture was diluted with HPLC-grade EtOAc (3 mL), satd. aqueous NH<sub>4</sub>Cl solution (3 mL) was added, the layers were separated and the aqueous was extracted with EtOAc (3 × 3 mL). The combined organic layers were washed with brine, dried (MgSO<sub>4</sub>), filtered and evaporated. The crude trihydroxy ester was dissolved in THF (125 μL) and H<sub>2</sub>O (250 μL) was added followed by LiOH·H<sub>2</sub>O (5.2 mg, 0.12 mmol) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 24 h when full conversion was indicated by TLC. The mixture was acidified with 1 M HCl to pH 2 and extracted with HPLC-grade EtOAc (5 × 2 mL). The crude product was adsorbed on silica gel and purified by column chromatography (silica gel, HPLC-grade hexane/EtOAc/MeOH gradient 10:1:0 to 0:10:1) furnishing racemic 18(RS)-18-F<sub>3t</sub>-IsoP (rac-1) (1.7 mg, 4.8 μmol, 78% over two steps) as a colorless oil and as partially separable 1.2:1 mixture of C-18 epimers as determined by <sup>1</sup>H NMR spectroscopy. The relative configuration at C-18 was assigned by comparing the <sup>1</sup>H and <sup>13</sup>C NMR spectra to those of enantiomerically enriched (R)-1 and (S)-1 (vide infra). **18(R\*)-epimer (major):** R<sub>f</sub> = 0.20 (EtOAc); <sup>1</sup>H NMR (600 MHz, CD<sub>3</sub>OD): δ = 5.56–5.52 (m, 2H), 5.45–5.29 (m, 4H), 3.97 (dt, <sup>3</sup>J<sub>H,H</sub> = 7.2, 5.0 Hz, 1H), 3.96–3.91 (m, 1H), 3.88 (dt, <sup>3</sup>J<sub>H,H</sub> = 7.8, 5.4 Hz, 1H), 2.83 (dt, <sup>2</sup>J<sub>H,H</sub> = 15.4 Hz, <sup>3</sup>J<sub>H,H</sub> = 8.1 Hz, 1H), 2.80 (dt, <sup>2</sup>J<sub>H,H</sub> = 15.4 Hz, <sup>3</sup>J<sub>H,H</sub> = 8.1 Hz, 1H), 2.73–2.62 (m, 1H), 2.487 (dt, <sup>2</sup>J<sub>H,H</sub> = 14.6 Hz, <sup>3</sup>J<sub>H,H</sub> = 7.4 Hz, 1H), 2.23–2.12 (m, 3H), 2.12–2.05 (m, 3H), 2.03 (quint, <sup>3</sup>J<sub>H,H</sub> = 6.8 Hz, 1H), 1.65 (quint, <sup>3</sup>J<sub>H,H</sub> = 7.6 Hz, 2H), 1.61–1.43 (m, 3H), 0.91 ppm (t, <sup>3</sup>J<sub>H,H</sub> = 7.5 Hz, 3H); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>): δ = 176.2, 135.2, 129.4, 129.0, 128.9, 128.7, 127.54, 76.9, 76.5, 74.04, 53.5, 50.4, 42.23, 32.6, 30.16, 27.2, 26.21, 25.9, 24.38, 9.67 ppm. **18(S\*)-epimer (minor):** R<sub>f</sub> = 0.25 (EtOAc 100%); <sup>1</sup>H NMR (600 MHz, CD<sub>3</sub>OD): δ = 5.52–5.49 (m, 2H), 5.45–5.29 (m, 4H), 3.98 (dt, <sup>3</sup>J<sub>H,H</sub> = 7.2, 5.0 Hz), 3.96–3.91 (m, 1H), 3.88 (dt, <sup>3</sup>J<sub>H,H</sub> = 7.8, 5.4 Hz, 1H), 2.78 (dt, <sup>2</sup>J<sub>H,H</sub> = 15.4 Hz, <sup>3</sup>J<sub>H,H</sub> = 8.1 Hz, 1H), 2.76 (dt, <sup>2</sup>J<sub>H,H</sub> = 15.4 Hz, <sup>3</sup>J<sub>H,H</sub> = 8.1 Hz, 1H), 2.73–2.66 (m, 1H), 2.491 (dt, <sup>2</sup>J<sub>H,H</sub> = 14.6 Hz, <sup>3</sup>J<sub>H,H</sub> = 7.4 Hz, 1H), 2.23–2.12 (m, 3H), 2.12–2.05 (m, 3H), 2.03 (quint, <sup>3</sup>J<sub>H,H</sub> = 6.8 Hz, 1H), 1.65 (quint, <sup>3</sup>J<sub>H,H</sub> = 7.6 Hz, 2H), 1.61–1.43 (m, 3H), 0.90 ppm (t, <sup>3</sup>J<sub>H,H</sub> = 7.5 Hz, 3H); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>): δ = 176.2, 135.2, 129.5, 129.1, 128.9, 128.8, 127.53, 76.9, 76.5, 74.07, 53.9, 50.4, 42.22, 32.5, 30.20, 27.1, 26.24, 25.9, 24.43, 9.72 ppm; IR (film): ν = 3624–2435 (v

br), 3352 (br), 3011, 2954, 2927, 2877, 1710, 1457, 1412, 1260, 1145, 1066, 1030, 1003, 970 cm<sup>-1</sup>; MS (+ESI) *m/z* (%): 375 (100) [M + Na]<sup>+</sup>, (-ESI) *m/z* (%): 351 (100) [M – H]<sup>-</sup>; HRMS (-ESI) *m/z*: calcd for C<sub>20</sub>H<sub>32</sub>O<sub>5</sub>·H<sup>+</sup>: 351.2177 [M – H]<sup>-</sup>, found: 351.2175; (+ESI) *m/z*: calcd for C<sub>20</sub>H<sub>32</sub>O<sub>5</sub> + Na<sup>+</sup>: 375.2142 [M + Na]<sup>+</sup>, found: 375.2140.

**18-F<sub>3t</sub>-IsoP ((S)-1):** HCl (3.5 mL, 0.5 N in MeOH, 1.75 mmol) was added to a solution of Wittig compound **21a** (100 mg, 0.175 mmol) in THF (20 mL) and MeOH (6.5 mL) at 0 °C. The reaction mixture was stirred at room temperature for 2.5 h until completion. After quenching by a spatula tip of NaHCO<sub>3</sub>, water (20 mL) was added and the mixture was extracted with EtOAc (3 × 20 mL), the combined organic layers were washed with brine, dried over MgSO<sub>4</sub> and the solvents were removed under reduced pressure. The crude product was purified by column chromatography (silica gel, EtOAc) giving 18-F<sub>3t</sub>-IsoP methyl ester (20 mg, 32%) as >95:5 diastereomeric mixture as determined by <sup>1</sup>H NMR spectroscopy as a colorless oil. R<sub>f</sub> = 0.36 (EtOAc); [α]<sub>D</sub><sup>20</sup> = +9.5 (c = 0.2 in MeOH); <sup>1</sup>H NMR (300 MHz, CD<sub>3</sub>OD): δ = 5.57–5.48 (m, 2H), 5.48–5.25 (m, 4H), 4.02–3.83 (m, 3H), 3.65 (s; 3H), 2.84–2.62 (m, 3H), 2.58–2.42 (dt, <sup>2</sup>J<sub>H,H</sub> = 14.4 Hz, <sup>3</sup>J<sub>H,H</sub> = 7.4 Hz, 1H), 2.33 (t, <sup>3</sup>J<sub>H,H</sub> = 7.4 Hz, 2H), 2.23–1.97 (m, 5H), 1.67 (quint, <sup>3</sup>J<sub>H,H</sub> = 7.6 Hz, 2H), 1.61–1.43 (m, 3H), 0.90 ppm (t, <sup>3</sup>J<sub>H,H</sub> = 7.4 Hz, 3H); <sup>13</sup>C NMR (75 MHz, CD<sub>3</sub>OD): δ = 175.8, 136.6, 130.6, 130.0, 129.9, 129.7, 129.6, 76.3, 76.2, 75.1, 53.8, 52.0, 51.3, 43.6, 34.1, 31.2, 27.51, 27.46, 26.7, 25.9, 10.3 ppm; MS (+ESI) *m/z*: 389 [M + Na]<sup>+</sup>; HRMS (+ESI): *m/z* calcd for: C<sub>21</sub>H<sub>34</sub>O<sub>5</sub> + Na<sup>+</sup>: 389.2298 [M + Na]<sup>+</sup>, found: 389.2305.

LiOH·H<sub>2</sub>O (14 mg, 0.33 mmol) was added to a solution of the above prepared 18-F<sub>3t</sub>-IsoP methyl ester (20 mg, 54 μmol) in THF (1 mL) and water (1 mL) and the reaction mixture was stirred at room temperature for 3 h. The reaction was quenched by slow addition of aqueous NaHSO<sub>4</sub> solution (1 M) until the pH was acidic. The mixture was extracted with EtOAc (3 × 2 mL), the combined organic layers were washed with brine (2 mL), dried over MgSO<sub>4</sub> and concentrated in vacuum. The crude product was purified by column chromatography (silica gel, EtOAc) affording 18-F<sub>3t</sub>-IsoP (S)-1 (7 mg, 37%) as a colorless oil. R<sub>f</sub> = 0.28 (EtOAc/MeOH 9:1); [α]<sub>D</sub><sup>20</sup> = +23.3 (c = 0.06 in MeOH); <sup>1</sup>H NMR (300 MHz, CD<sub>3</sub>OD): δ = 5.54–5.49 (m, 2H), 5.46–5.31 (m, 4H), 4.01–3.83 (m, 3H), 2.87–2.76 (m, 2H), 2.76–2.63 (m, 1H), 2.49 (dt, <sup>2</sup>J<sub>H,H</sub> = 14.2 Hz, <sup>3</sup>J<sub>H,H</sub> = 7.3 Hz, 1H), 2.30 (t, <sup>3</sup>J<sub>H,H</sub> = 7.4 Hz, 2H), 2.21–1.97 (m, 5H), 1.66 (quint, <sup>3</sup>J<sub>H,H</sub> = 7.3 Hz, 2H), 1.60–1.42 (m, 3H), 0.91 ppm (t, <sup>3</sup>J<sub>H,H</sub> = 7.5 Hz, 3H); <sup>13</sup>C NMR (75 MHz, CD<sub>3</sub>OD): δ = 177.4, 136.6, 130.6, 130.00, 129.98, 129.7, 76.3, 76.2, 75.1, 53.8, 51.3, 43.5, 34.4, 31.2, 27.6, 27.5, 26.7, 26.0, 10.3 ppm; MS (-ESI) *m/z*: 351 [M – H]<sup>-</sup>; HRMS (-ESI): *m/z* calcd for C<sub>20</sub>H<sub>32</sub>O<sub>5</sub>·H<sup>+</sup>: 351.2177 [M – H]<sup>-</sup>, found: 351.2172.

**20-F<sub>4t</sub>-NeuroP ((S)-2):** 20-F<sub>4t</sub>-NeuroP methyl ester (25.6 mg, 65%) was prepared in analogy to 18-F<sub>3t</sub>-IsoP methyl ester from ester **22a** (73.7 mg, 0.1 mmol) as a colorless oil of >95:5 diastereomeric mixture (determined by <sup>1</sup>H NMR spectroscopy). R<sub>f</sub> = 0.11 (EtOAc); [α]<sub>D</sub><sup>20</sup> = –7.0 (c = 0.2 in CHCl<sub>3</sub>); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ = 5.62–5.43 (m, 2H), 5.43–5.26 (m, 6H), 4.06–3.85 (m, 3H), 3.65 (s; 3H), 3.00 (br s; 1H), 2.89–2.62 (m, 5H), 2.58–2.26 (m, 6H), 2.19–2.06 (m, 1H), 2.06–1.96 (m, 2H), 1.91 (br s; 1H), 1.69–1.41 (m, 3H), 0.87 ppm (t, <sup>3</sup>J<sub>H,H</sub> = 7.4 Hz, 3H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): δ = 173.9, 135.9, 129.7, 129.3, 129.1, 128.5, 128.2, 128.0, 127.8, 76.3, 76.2, 74.3, 53.5, 51.7, 50.8, 42.3, 34.0, 30.1, 27.0, 25.8, 25.6, 22.8, 9.8 ppm; MS (+ESI) *m/z*: 375 [M + H – H<sub>2</sub>O]<sup>+</sup>; HRMS (+ESI): *m/z* calcd for C<sub>23</sub>H<sub>36</sub>O<sub>5</sub> + H<sup>+</sup>·H<sub>2</sub>O: 375.2530 [M + H – H<sub>2</sub>O]<sup>+</sup>, found: 375.2533.

20-F<sub>4t</sub>-NeuroP (34.2 mg, 63%) was prepared in analogy to (S)-1 from 20-F<sub>4t</sub>-NeuroP methyl ester (55 mg, 0.14 mmol) as a colorless oil. R<sub>f</sub> = 0.48 (EtOAc/HCOOH 9:1); [α]<sub>D</sub><sup>20</sup> = +12.6 (c = 0.5 in MeOH); <sup>1</sup>H NMR (300 MHz, CD<sub>3</sub>OD): δ = 5.58–5.47 (m, 2H), 5.45–5.22 (m, 6H), 4.00–3.79 (m, 3H), 2.89–2.73 (m, 4H), 2.71–2.61 (m, 1H), 2.47



(quint,  $^3J_{\text{H,H}}=7.4$  Hz, 1H), 2.40–2.23 (m, 4H), 2.23–1.98 (m, 3H), 1.61–1.39 (m, 3H), 0.91 ppm (t,  $^3J_{\text{H,H}}=7.4$  Hz, 3H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta=177.1, 136.6, 130.6, 130.2, 129.8, 129.6, 129.3, 129.1, 76.3, 76.2, 75.1, 53.8, 51.3, 43.5, 35.1, 31.2, 27.5, 26.7, 26.5, 23.9, 10.3$  ppm; MS (+ESI)  $m/z$ : 401  $[\text{M}+\text{Na}]^+$ ; HRMS (+ESI):  $m/z$  calcd for  $\text{C}_{22}\text{H}_{34}\text{O}_5 + \text{Na}^+$ : 401.2300  $[\text{M}+\text{Na}]^+$ , found: 401.2301.

**20-F<sub>3t</sub>-NeuroP ((S)-3)**: 20-F<sub>3t</sub>-NeuroP methyl ester (25 mg, 93%) was prepared in analogy to 18-F<sub>3t</sub>-IsoP methyl ester from silylated ester **23a** (50 mg, 0.068 mmol) as >95:5 diastereomeric mixture (determined by  $^1\text{H}$  NMR spectroscopy) as a colorless oil.  $[\alpha]_{\text{D}}^{20}=+2.2$  ( $c=0.2$  in MeOH);  $^1\text{H}$  NMR (300 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta=5.60\text{--}5.49$  (m, 2H), 5.47–5.26 (m, 4H), 3.96–3.63 (m, 3H), 3.65 (s; 3H), 2.85–2.65 (m, 3H), 2.49 (dt,  $^2J_{\text{H,H}}=14.6$  Hz,  $^3J_{\text{H,H}}=7.4$  Hz, 1H), 2.32 (t,  $^3J_{\text{H,H}}=7.4$  Hz, 2H), 2.17–1.73 (m, 5H), 1.66–1.42 (m, 5H), 1.38–1.30 (m, 4H), 0.91 ppm (t,  $^3J_{\text{H,H}}=7.4$  Hz, 3H); MS (+ESI)  $m/z$ : 417  $[\text{M}+\text{Na}]^+$ ; HRMS (+ESI):  $m/z$  calcd for  $\text{C}_{23}\text{H}_{38}\text{O}_5 + \text{Na}^+$ : 417.2611  $[\text{M}+\text{Na}]^+$ , found: 417.2617.

20-F<sub>3t</sub>-NeuroP (S)-3 (15 mg, 60%) was prepared in analogy to (S)-1 from 20-F<sub>3t</sub>-NeuroP methyl ester (26 mg, 0.066 mmol) as a colorless oil.  $[\alpha]_{\text{D}}^{20}=+2.7$  ( $c=0.2$  in MeOH);  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta=5.57\text{--}5.47$  (m, 2H), 5.44–5.34 (m, 4H), 3.98–3.86 (m, 3H), 2.85–2.68 (m, 3H), 2.53–2.44 (m, 1H), 2.28 (t,  $^3J_{\text{H,H}}=7.4$  Hz, 2H), 2.18–2.04 (m, 5H), 1.68–1.42 (m, 5H), 1.41–1.30 (m, 4H), 0.91 ppm (t,  $^3J_{\text{H,H}}=7.4$  Hz, 3H);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta=176.8, 136.6, 130.6, 130.9, 129.8, 129.6, 129.1, 76.3, 76.2, 75.1, 53.8, 51.3, 43.6, 35.2, 31.3, 30.5, 29.9, 28.1, 27.5, 26.8, 26.1, 10.3$  ppm; MS (-ESI)  $m/z$ : 379  $[\text{M}-\text{H}]^-$ ; HRMS (-ESI) calcd for  $\text{C}_{22}\text{H}_{36}\text{O}_5-\text{H}^-$ : 379.2490  $[\text{M}-\text{H}]^-$ , found: 379.2484.

## Identification of DHA and EPA metabolites in edible oils

**Oil from food supplement capsules sampling**: Food supplement capsules were delivered from their respective manufacturers. Capsules „UnoCardio® 1000 + Vitamin D1000“ from WHC Health Consulting, ingredients: rTriglyceride (95% Omega-3; 1200 mg) EPA (675 mg), DHA (460 mg), vitamin D<sub>3</sub> (1000 UI). Capsules „NourSea Calanus® oil“ from NourSea Calanus®, ingredients: sea natural plankton lipid extract of calanus oil (*Calanus finmarchicus*, 500 mg) containing wax esters (425 mg); among these unsaturated fatty acids (145 mg) including omega-3 PUFA (105 mg), astaxanthin (300 µg). Capsules were incised using a needle and the oily content was extracted by simple pressure of fingers into a glass vessel.

**Extraction of lipids**: After lipid extraction, oxidative damage was measured by determining the isoprostanoic acid levels in oily extracts based on micro-LC-MS/MS. Note that each type of sample has been subject to a technical triplicate (e.g. three capsules for each edible oil).

Briefly, the oily extract (250 mg) was mixed with hexane (5 mL), MeOH (2 mL), internal standards (IS; mixture 1/1/1 of deuterium-isotopic d<sub>4</sub>-10(R)-10F<sub>4t</sub>-NeuroP/d<sub>4</sub>-15-F<sub>2t</sub>-IsoP and C<sub>21</sub>-15-F<sub>2t</sub>-IsoP/C<sub>19</sub>-16-F<sub>1t</sub>-PhytoP, 4 ng) and the mixture was vortexed for 15 s. The material was supplemented with formic acid (40 mM, pH 4.6; 2 mL) before centrifugation at 2000 rpm at room temperature for 5 min. The supernatant was separated and the lower phase was loaded on cartridges (SPE Oasis MAX, 3cc, 60 mg, Waters, Milford, MA, USA) previously conditioned by MeOH (2 mL) and equilibrated with formic acid (20 mM, pH 4.5; 2 mL). The cartridges were successively washed with: aqueous NH<sub>3</sub> (2 vol.%; 2 mL), formic acid (20 mM)/MeOH (70:30; 2 mL), hexane (2 mL) and hexane/ethyl acetate (70:30; 2 mL). Targeted metabolites were eluted with hexane/ethanol/acetic acid (70:29.4:0.6; 2 mL). The eluates were concentrated to dryness under a nitrogen flow at 40 °C. Solutions were reconstituted by addition of aqueous formic acid (0.1 vol.%) / CH<sub>3</sub>CN

(83:17; 100 µL) prior to injection in the microLC-QTRAP® system. Hexane, NH<sub>4</sub>OH, EtOAc, formic acid, and EtOH were HPLC grade chemicals. MeOH, H<sub>2</sub>O, acetic acid and CH<sub>3</sub>CN were LCMS grade chemicals.

## Liquid chromatography tandem mass spectrometry analysis:

Oxidized lipid products were analyzed according to the method previously reported.<sup>[20]</sup> Briefly, samples were analyzed using a micro liquid chromatography (Eksigent® MicroLC 200 plus; Eksigent Technologies, CA, USA) equipped with an autosampler (CTC Analytics AG Zwingen, Switzerland). The autosampler vial tray was kept at 10 °C and the column (HALO C18 100×0.5 mm, 2.7 µm, Eksigent Technologies, CA, USA) was set at 40 °C. The mobile phase consisted of a binary gradient of solvent A (aqueous formic acid, 0.1% v/v) and solvent B (CH<sub>3</sub>CN/MeOH 8:2). The gradient elution program was performed as follows: 17% solvent B at 0 min until 1.6 min, then 21% solvent B at 2.8 min, 25% solvent B at 7.3 min, 28.5% solvent B at 8.8 min, 33.1% solvent B at 9.6 min, 33.3% solvent B at 10.9 min, 40% solvent B at 15 min and 95% solvent B at 16.5 min for 2.5 min and returned to the initial conditions. The sample (5 µL) was injected and eluted at a flow rate of 0.03 mL min<sup>-1</sup>. A SCIEX QTRAP® 5500 mass spectrometer (Sciex Applied Biosystems) was used to analyze isoprostanoic acids. The ionization source was an electrospray (ESI), operated in the negative mode. The source voltage was kept at -4.5 kV and nitrogen was used as curtain gas. MRM analysis was conducted by monitoring transitions from the precursor ion. Data processing was performed by MultiQuant 3.0 software (Sciex Applied Biosystems). Isoprostanoic acid quantification was based on the analyte to IS ratio using external calibration curves, which were calculated by linear regression of at least six standard concentrations. All data were expressed in relative percentage of the analytes because we were unable to perform spiked experiments in order to calculate matrix effect and extraction yield.

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## Conflict of interest

The authors declare no conflict of interest.

**Keywords:** isoprostanes • neuroprostanes • PUFA metabolites • quantification • total synthesis

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