Stereoselective synthesis of MaR2$_{n\text{-}3\ DPA}$

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Abstract: The first total synthesis of the n-3 docosapentaenoic derived oxygenated product MaR2$_{n\text{-}3\ DPA}$ has been achieved. The 13*R* and 14*S* stereogenic centers were introduced using 2-deoxy-*D*-ribose in a chiral pool strategy. The geometry of the *Z*,*E*,*E*-triene moiety was prepared using highly *E*-selective Wittig- and Takai-olefination reactions as well as the *Z*-stereoselective Lindlar reduction. LC/MS-MS data of synthetic MaR2$_{n\text{-}3\ DPA}$ matched data for the biosynthetic formed product that enabled the configurational assignment of this oxygenated natural product to be (7*Z*,9*E*,11*E*,13*R*,14*S*,16*Z*,19*Z*)-13,14-dihydroxydocosa-7,9,11,16,19-pentaenoic acid.

Keywords: maresins; MaR2$_{n\text{-}3\ DPA}$; n-3 docosapentaenoic acid; specialized pro-resolving mediator.

Recent studies have demonstrated that polyunsaturated fatty acids (PUFAs) derived specialized pro-resolving mediators (SPMs) actively govern and promote the resolution of inflammation.[1] PUFAs are enzymatically converted into different families of SPMs, e.g. the lipoxins, resolvins, protectins and maresins.[2] Maresin 1 (MaR1) is biosynthesized[3] from

docosahexaenoic acid (DHA) in the presence of 12-lipoxygenase and was the first member of the maresin family of SPMs to be reported[4] and prepared by total synthesis.[5]

In 2013 Dalli and co-workers reported several new SPMs biosynthesized from n-3 docosapentaenoic acid (n-3 DPA).[6] n-3 DPA, consisting of 22 carbons and five all-*Z* double bonds, is an elongated product of eicosapentaenoic acid and an intermediate in the biosynthesis of DHA.[7] Using a self-limited model of inflammation and targeted metabololipidomics during the onset and resolution of acute inflammation, Dalli and co-workers[6] uncovered several novel n-3 DPA SPMs that are potent bioactive molecules. The structures of $MaR1_{n\text{-}3\ DPA}$ (**1**), $MaR2_{n\text{-}3\ DPA}$ (**2**) and $MaR3_{n\text{-}3\ DPA}$ (**3**) are shown in Figure 1.

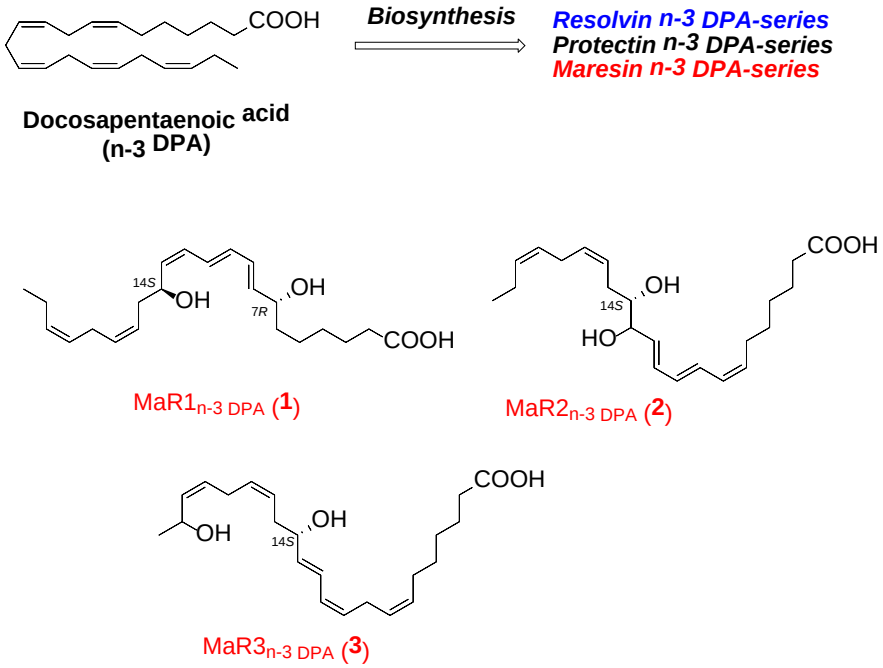
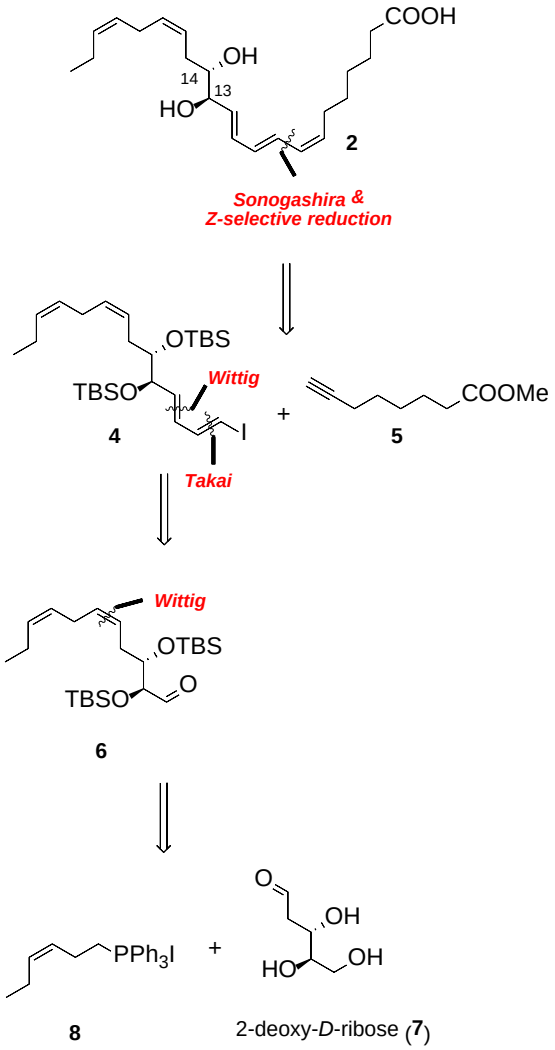


Figure 1. Structures of $MaR1_{n\text{-}3\ DPA}$ (**1**), $MaR2_{n\text{-}3\ DPA}$ (**2**) and $MaR3_{n\text{-}3\ DPA}$ (**3**). The absolute configuration is presented where established.

Based on their novel pro-resolving and anti-inflammatory bioactions, SPMs have attracted significant interest from the biomedical, pharmacological and synthetic organic communities.[8] SPMs act as agonists on individual GPCRs[9] exhibiting nanomolar pro-resolution and anti-inflammatory bioactions.[10] Some SPMs have entered initial clinical trial development programs.[11] These endogenously formed products are available in minute amounts from their natural sources and contain several stereogenic centers and conjugated *E*- and *Z*-double bonds. Hence, stereoselective synthesis for configurational assignment and extensive biological testing becomes necessary.

A few of the n-3 DPA-derived SPMs have recently been prepared[12] and subjected to biological evaluations,[13] but MaR2$_{n\text{-}3\ DPA}$ (**2**) has not been synthesized to date and its absolute configuration at C-13 remained to be determined. These facts, as well as the high demand for sufficient material for biological and pharmacological testing, inspired us to report the first total synthesis of MaR2$_{n\text{-}3\ DPA}$ (**2**).

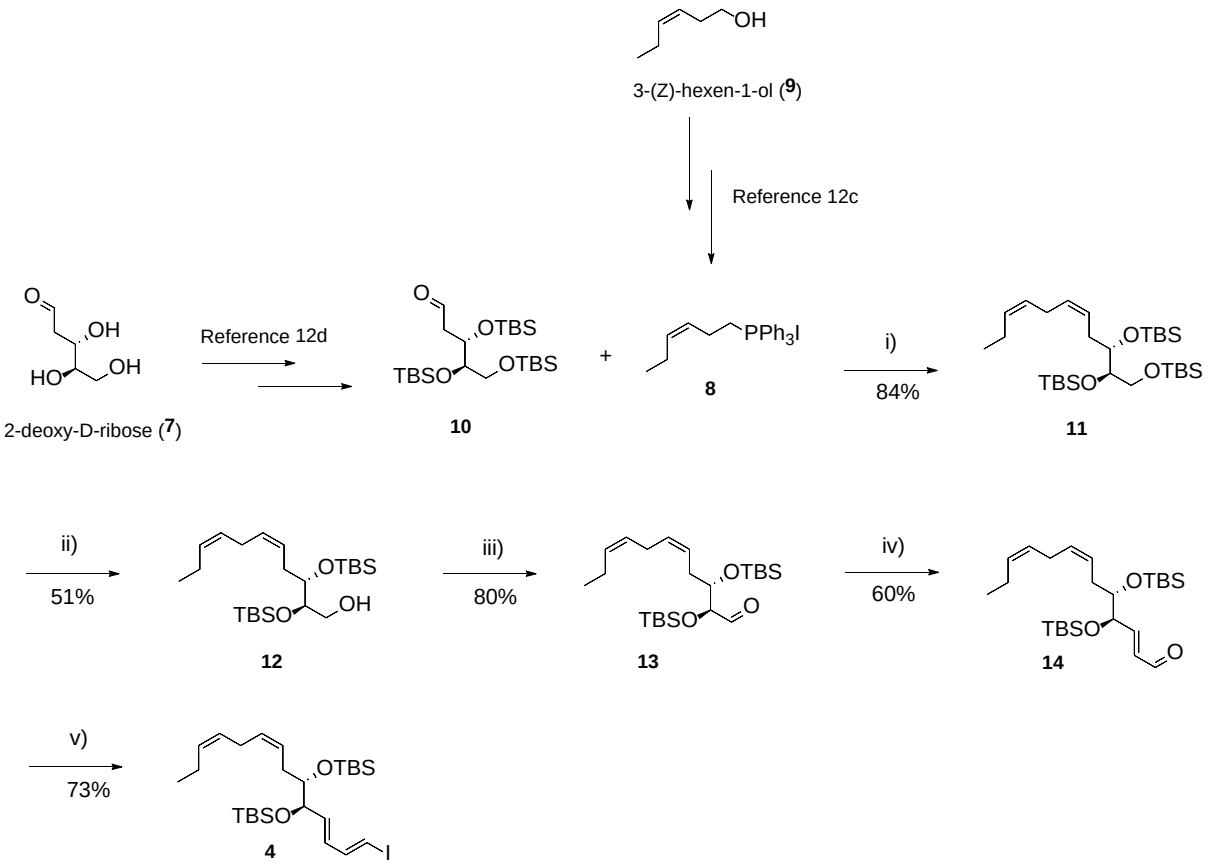
The three key intermediates **4**, **5** and **6** in our retrosynthetic analysis are depicted in Scheme 1. The stereogenic centers at C13 and C14 were assumed to be *R* and *S*, respectively, based on biosynthetic considerations.[6] Hence, 2-deoxy-*D*-ribose (**7**) was deemed a suitable commercially available starting material for preparing MaR2$_{n\text{-}3\ DPA}$ (**2**). This carbohydrate has been used in the stereoselective total synthesis of other SPMs.[14]



Scheme 1. Retrosynthetic analysis of MaR2$_{n\text{-}3\ DPA}$ (**2**).

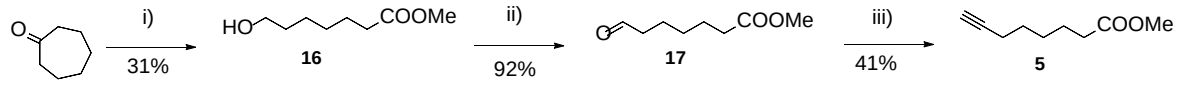
The phosphonium salt **8** was synthesized from *Z*-hex-3-en-1-ol (**9**) as previously

described.[12c] Intermediate **11** was obtained from known TBS-protected aldehyde **10**[12d] using a highly *Z*-selective Wittig reaction with the *in situ* generated ylide of **8** (Scheme 2). This produced **11** as one diastereomer in 84% yield (ESI). Next, a selective deprotection of the primary TBS-group in **11** was achieved with *para*-toluene sulfonic acid (PTSA) in MeOH at -20 °C giving alcohol **12** that was oxidized (Dess-Martin periodinane (DMP), $NaHCO_3$, CH_2Cl_2) to its aldehyde **13** in 40% yield over the two steps. Aldehyde **13** was dissolved in toluene and 1.3 equiv. of the stabilized ylide (triphenyl-phosphoranylidene)acetaldehyde was added. The reaction mixture was heated at reflux for 19 hours to afford the *E*-configured α,β-unsaturated aldehyde **14** in 60% yield after purification by column chromatography (ESI). To complete the formation of fragment **4**, a Takai reaction was performed on aldehyde **14**. After acidic work-up and purification by column chromatography the sensitive *E,E*-vinylic iodide **4** was isolated in 73% yield (Scheme 2).



Scheme 2. Synthesis of vinylic iodide **4**. Reagents and conditions: i) NaHMDS, CH_2Cl_2, - 78 °C; ii) *para*-toluene sulfonic acid (PTSA), MeOH, -20 °C; iii) DMP, $NaHCO_3$, CH_2Cl_2; iv) toluene, (triphenyl-phosphoranylidene)acetaldehyde, Δ; v) $CrCl_2$, dioxane, THF, CHI_3, 0 °C.

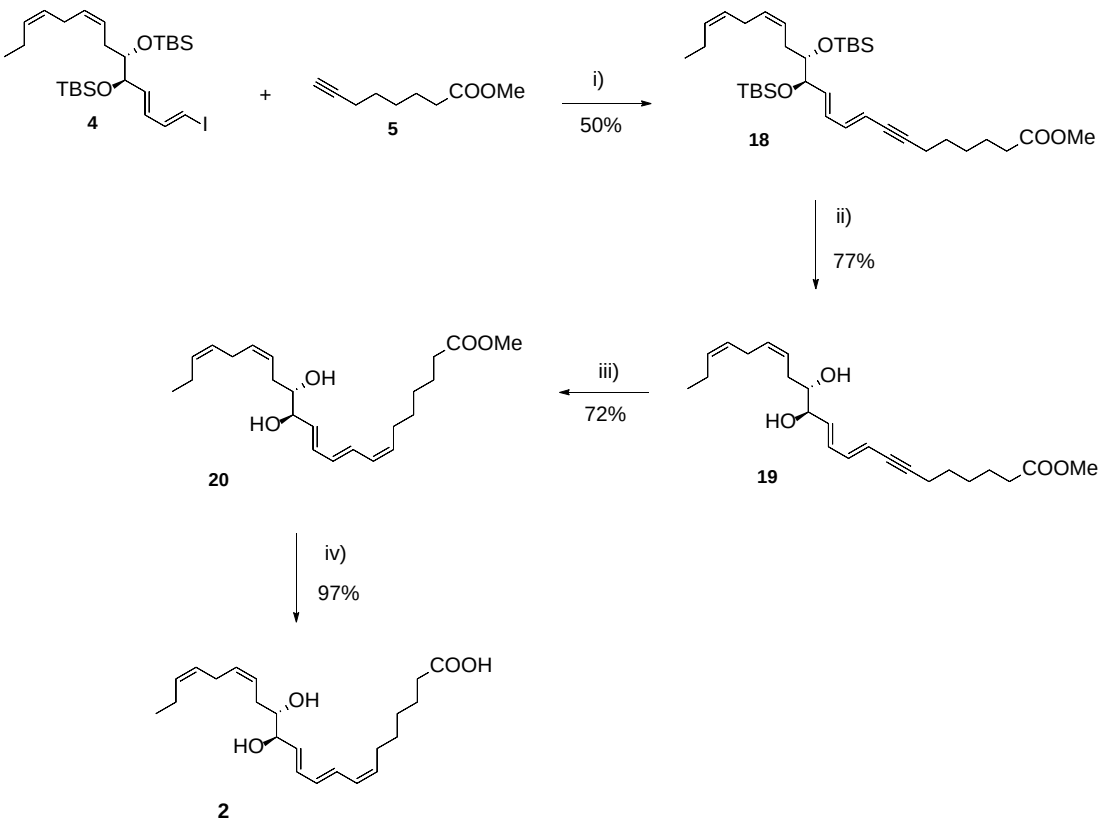
Terminal alkyne **5** was conveniently prepared in a four-step sequence, starting from cycloheptanone (**15**), see Scheme 3. Bayer-Villiger oxidation on **15** followed by Fischer-esterification gave hydroxyl-ester **16** that was oxidized to aldehyde **17** and reacted in the Ohira-Bestmann reaction affording alkyne **5** in 12% yield from **15**.



cycloheptanone (**15**)

Scheme 3. Synthesis of alkyne **5**. Reagents and conditions: i) a) *m*-CPBA, CH_2Cl_2; b) MeOH, H_2SO_4; ii) DMP, $NaHCO_3$, CH_2Cl_2; iii) dimethyl(1-diazo-2-oxopropyl) phosphonate; K_2CO_3, MeOH.

The Sonogashira coupling reaction with key fragments **4** and **5** produced alkyne **18** in 50% isolated yield after careful chromatographic purification (Scheme 4). Next, removal of the two-TBS groups in **18** with excess TBAF in THF produced diol **19**. Reduction of the internal alkyne in **19** using the Lindlar-reduction (Pd-$CaCO_3$, EtOAc/pyridine/1-octene, H_2 1 atm) gave the methyl ester of $MaR2_{n\text{-}3\ DPA}$ (**20**) in 55% isolated yield over the two steps and with > 95% chemical purity (HPLC, ESI). Finally, careful saponification (LiOH, H_2O, MeOH, 0 ^oC) of **20** gave $MaR2_{n\text{-}3\ DPA}$ (**2**) in 97% yield (Scheme 4). Data from NMR, LC/MS-MS and UV experiments (ESI) confirmed the structure of **2**.



Scheme 4. Total synthesis of MaR2$_{n\text{-}3\ DPA}$ (**2**). Reagents and conditions: i) CuI, Et_2NH, $Pd(PPh_3)_4$ (5%); ii) TBAF, THF; iii) $Pd/CaCO_3$, EtOAc/pyridine/1-octene, H_2; iv) LiOH, H_2O, MeOH, 0 ^oC.

We next tested whether synthetic **2** matched the endogenous MaR2$_{n\text{-}3\ DPA}$ (**2**) prepared from human samples. We first isolated material from human serum and the retention time of the endogenous mediator using RP-HPLC-MS-MS lipid mediator profiling experiments.[15] Using multiple reaction monitoring (MRM) of the ~~on~~ parent ion with *m/z* 361 and the daughter ions *m/z* 223 or *m/z* 193, we obtained a sharp peak with retention time (R_T) of 14.4 min (Fig. 2A). Of note, a similar retention time of 14.4 min was obtained with synthetic **2** (see Fig. 2A). Moreover, co-injection (2 μL) of a homogenous sample of biological MaR2$_{n\text{-}3\ DPA}$ (**2**) with synthetic **2** in a 1:10 molar ratio, respectively, gave a single sharp peak in MRM experiments, with R_T 14.4 min (Fig. 2A). Similar findings were made with platelet rich plasma, where endogenous MaR2$_{n\text{-}3\ DPA}$ (**5**) gave a R_T of 14.4 min that co-eluted with synthetic **2** (Fig. 2B). To obtain further evidence that the chemical structure for synthetic **2** matches that of endogenous MaR2$_{n\text{-}3\ DPA}$ we next assessed the MS/MS fragmentation spectra. Here we found that, in accordance with published findings,[6] MaR2$_{n\text{-}3\ DPA}$ from both human serum and platelet rich plasma gave the following ions *m/z* 361 = M-H, *m/z* 344 = M-H-H_2O,

m/z 325 = M-H-2H_2O, *m/z* 317 = M-H-CO_2, *m/z* 299 = M-H-H_2O-CO_2, *m/z* 281 = M-H-2H_2O-CO_2, *m/z* 179 = 223-CO_2, *m/z* 161 = 223-H_2O-CO_2, *m/z* 149 = 193-CO_2, ions that were also found in the MS/MS spectrum of synthetic **2** (Fig. 3).

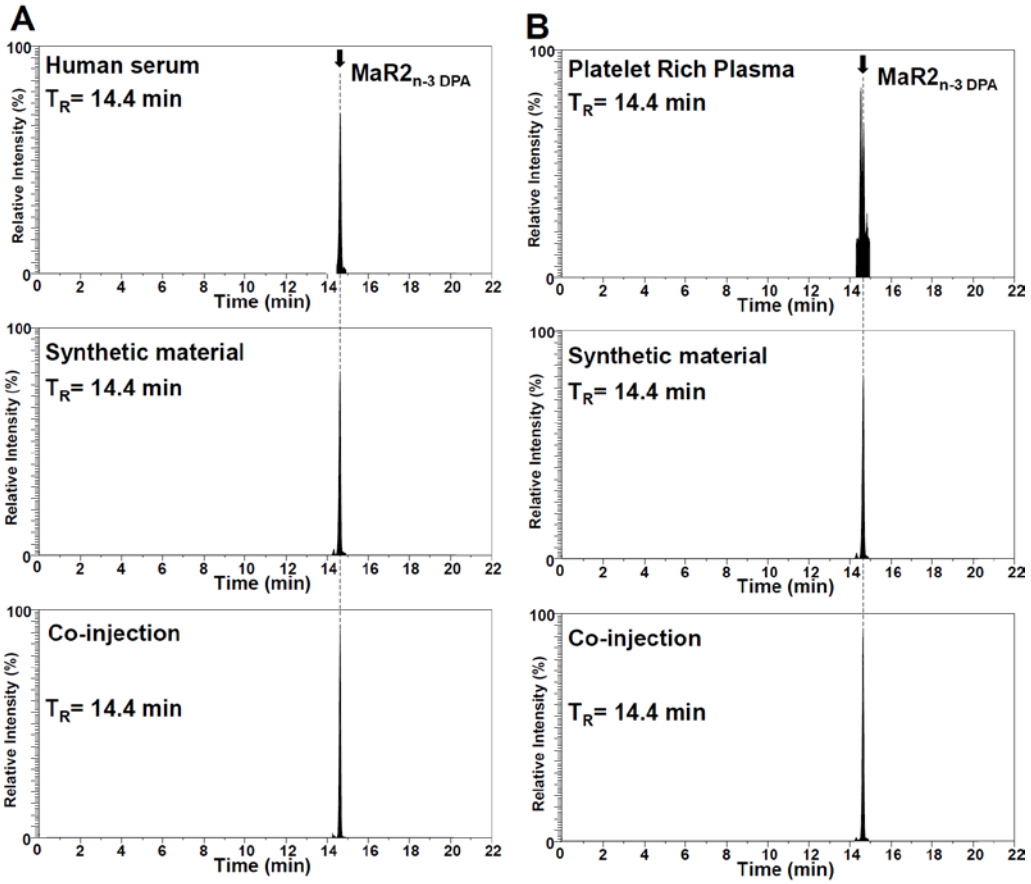


Figure. 2. Synthetic **2** matches endogenous MaR2$_{n-3\ DPA}$ in human serum and cells. (A) human serum (B) platelet rich plasma were collected, placed in ice-cold methanol, lipid mediators were extracted and MaR2$_{n-3\ DPA}$ was identified using lipid mediator profiling. Panels depict representative MRM chromatograms for *m/z* 361>223 (human serum) or *m/z* 361>193 (platelet rich plasma). Top panels depict the chromatograms obtained with biological material, center panels depict chromatograms obtained with synthetic **2** and bottom panels depict chromatograms obtained with the biological material co-injected with synthetic **2**. Results are representative of three determinations for A and n = 3 distinct human donors for B.

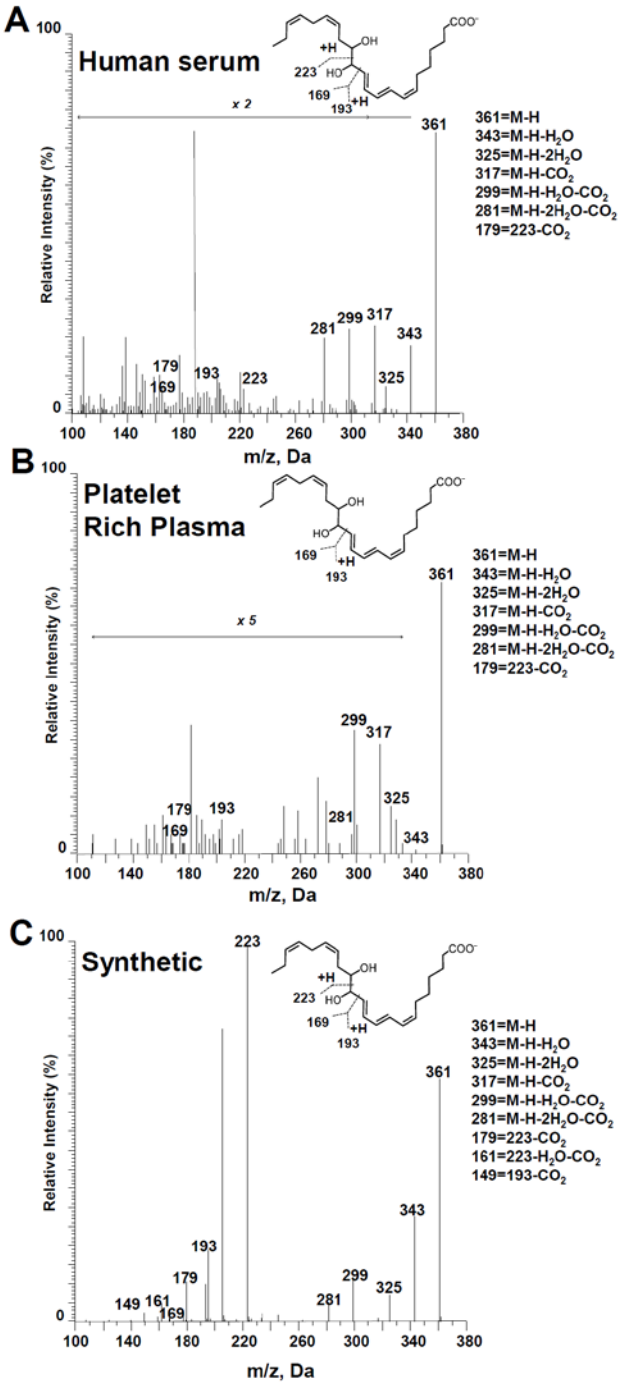


Figure 3. MS/MS fragmentation spectra for synthetic **2** and $MaR2_{n\text{-}3\ DPA}$ from human serum and platelet rich plasma. Lipid mediators were extracted from (A) human serum and (B) platelet rich plasma and MS/MS spectra for endogenous $MaR2_{n\text{-}3\ DPA}$, together with those of (C) synthetic **2**, were obtained using lipid mediator profiling. Results are representative of n=3 determination for A and C and 3 volunteers for B.

The SPMs are among the most exciting small and naturally occurring molecules currently undergoing investigations towards drug development of new anti-inflammatory drugs.[1,16] The

stereoselective synthesis of **2** using the Lindlar reaction, the Sonogashira coupling reaction and the Takai olefination produced multi milligram quantities of **2** that is now available for further biological and pharmacological evaluations to be conducted.

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Supplementary Material

Copies of ^{1}H and ^{13}C NMR spectra for intermediates and characterization data (UV/VIS spectra, HPLC chromatograms and LC/MS-MS spectra from matching experiments) of **2** as well as experimental procedures, are available at **[to be inserted by editorial office]**.