Stereocontrolled Synthesis and Investigation of the biosynthetic transformations of 16(*S*),17(*S*)-epoxy-$PD_{n\text{-}3\ DPA}$

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Abstract

$PD1_{n\text{-}3\ DPA}$ is a specialized pro-resolving lipid mediator that displays potent anti-inflammatory properties and pro-resolving bioactivities. Such naturally occurring compounds are of current interest in biomolecular chemistry and drug discovery. To investigate the involvement of an epoxide intermediate in the biosynthesis of $PD1_{n\text{-}3\ DPA}$ from n-3 docosapentaenoic acid, the epoxy acid 16(*S*),17(*S*)-epoxy-$PD_{n\text{-}3\ DPA}$, herein named $ePD_{n\text{-}3\ DPA}$, was prepared by stereoselective total synthesis. The synthetic material of $ePD_{n\text{-}3\ DPA}$ allowed investigations of its role in the biosynthesis of $PD1_{n\text{-}3\ DPA}$. The obtained results establish that the biosynthesis of $PD1_{n\text{-}3\ DPA}$ in neutrophils occurs with $ePD_{n\text{-}3\ DPA}$ as the intermediate, and that 15-LOX produces $ePD_{n\text{-}3\ DPA}$ from n-3 docosapentaenoic acid. Furthermore, support for the involvement of a hydrolytic enzyme in the biosynthetic conversion of $ePD_{n\text{-}3\ DPA}$ to $PD1_{n\text{-}3\ DPA}$ was found. In addition, $ePD_{n\text{-}3\ DPA}$ was found to regulate the formation of the potent neutrophil chemoattractant LTB_4 with equal potencies to that obtained with $PD1_{n\text{-}3\ DPA}$.

Introduction

The resolution of inflammation is an active process orchestrated by specialized pro-resolving lipid mediators (SPMs).[1] Failure of effective resolution may lead to tissue injury and the development of numerous human diseases. These compounds are biosynthesized from polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA, 20:5), docosahexaenoic acid (DHA, 22:6) and n-3 docosapentaenoic acid (n-3 DPA, 22:5) in the presence of cyclooxygenase and lipoxygenase enzymes. Over the last two decades it has been demonstrated that the resolution of inflammation and return to homeostasis are strictly controlled by the biosynthesis of several families of SPMs.[2] The SPMs exhibit potent anti-inflammatory and pro-resolving bioactions in the low nanomolar range as agonists towards G-protein coupled receptors (GPCRs).[3] These actively controlled resolution processes provide vast possibilities for drug development research.[4] Hence, the SPMs have attracted a great interest from the biomedical, pharmacological and synthetic organic communities.[5]

The first SPMs identified were the E-series resolvins,[6] but later the D-series resolvins,[7] the protectins[7-8] and the maresins,[9] as well as the sulfido-conjugates RCTRs (resolvin conjugates

in tissue regeneration), PCTR (protectin conjugates in tissue regeneration) and MCTRs (maresin conjugates in tissue regeneration)[10] have been reported. Some examples are depicted in Figure 1.

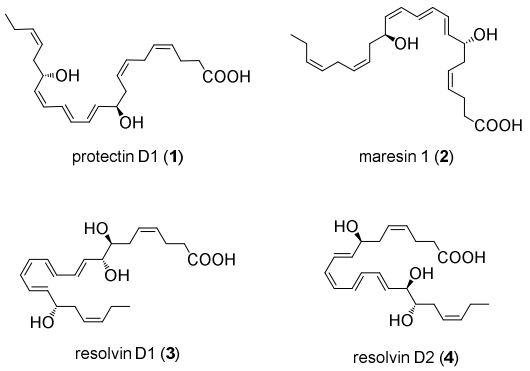


Figure 1. Examples of SPMs derived from DHA.

More recently, new SPMs biosynthesized from the PUFA n-3 DPA were uncovered.[11] N-3 DPA is an intermediate in the biosynthesis of DHA from EPA.[12] $PD1_{n\text{-}3\ DPA}$ (**5**) was one of the first n-3 DPA derived SPMs to be reported (Figure 2). The first total synthesis of **5** confirmed the configuration shown in Figure 2.[13]

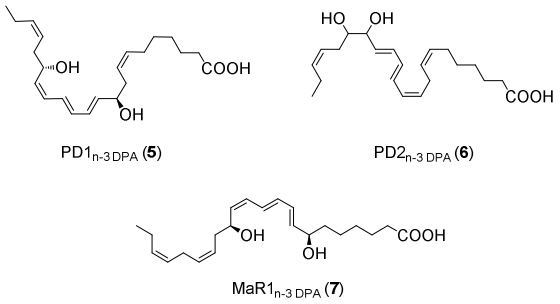
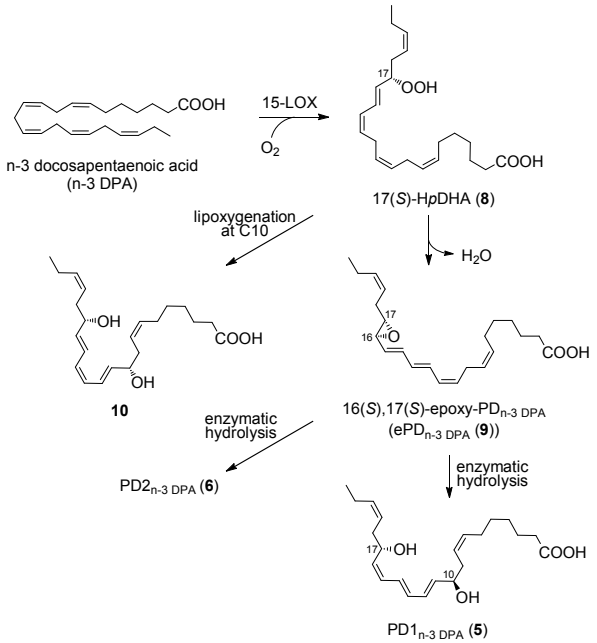


Figure 2. Examples of SPMs derived from n-3 DPA.

The natural product **5** demonstrated potent anti-inflammatory properties together with pro-resolving actions. Administration of 10 ng of synthetic **5** per mouse significantly reduced neutrophil recruitment during peritonitis.[13] Compound **5** also stimulated human macrophage phagocytosis and efferocytosis,[13] both key processes in the resolution of inflammation.[2] The chemistry and biology of the n-3 DPA-derived mediators has recently been reviewed.[14]

A proposed biosynthesis of **5** was disclosed in the original publication by Dalli *et al.*[11a] The biosynthesis was suggested to occur through a 17-lipoxygenation of n-3 DPA to 17(*S*)-hydroperoxy-7*Z*,10*Z*,13*Z*,15*E*,19*Z*-docosapentaenoic acid (17(*S*)-H*p*DPA, **8**), which is then further converted into the putative intermediate 16(*S*),17(*S*)-epoxy $PD_{n\text{-}3\ DPA}$ (**9**), herein named

ePD1$_{n\text{-}3\ DPA}$, see Scheme 1. The authors then suggested that enzymatic hydrolysis of epoxy acid **9** gives **5** and PD2$_{n\text{-}3\ DPA}$ (**6**). Of note, a lipoxygenation process from **8** produces 10(*S*),17(*S*)-dihydroxy-7*Z*,11*E*,13*Z*,15*E*,19*Z*-docosapentaenoic acid (**10**).

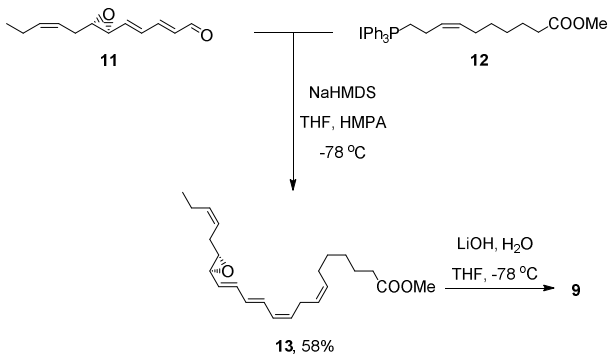


Scheme 1. Proposed biosynthesis of **5** and isomers.

Epoxides have earlier been reported as intermediates in the biosynthesis of oxygenated PUFAs, including SPMs derived from EPA and DHA.[5] The E-series and D-series resolvins are both biosynthesized via epoxide intermediates produced by human leukocytes confirmed by using acid alcohol trapping and $^{18}O_2$ incorporation.[7, 15] Serhan, Petasis and co-workers reported that the DHA derived SPM maresin 1 (**2**) is also biosynthesized *via* an epoxide intermediate.[16] Recently the biosynthesis of protectin D1 (**1**)[17] was established based on the initial studies reported by Serhan and co-workers.[18] It should be expected that the biosynthesis of the two SPMs **1** and **5** would occur in a similar fashion, given that the compounds are congeners. The only structural difference between these two SPMs is the absence of the C4-C5 *cis*-configured double bond in **5**. Hence, we became interested in preparing **9** by total synthesis and subject the synthetic material to biological systems to investigate the involvement of this epoxide in the biosynthesis of **5** from n-3 DPA. In addition, we wanted to investigate whether synthetic **9** regulates LTB_4 biosynthesis in human neutrophils, as LTB_4 production is associated with inflammatory processes.

Results and discussion

To obtain stereochemically pure **9**, we first prepared the two known fragments **11** and **12** as previously reported,[17, 19] see Scheme 2. The two fragments were then merged in a *Z*-selective Wittig-reaction. After chromatography, stereochemically pure **13** was obtained in 58% yield. The chemical purity and stereochemical integrity of **13** was validated using NMR analyses. The unambiguous assignment of the geometrical configurations of the olefins constituting the *E,E,Z*-triene moiety was performed using ^{1}H and ^{1}H-^{1}H COSY-45 NMR experiments (Electronic Supporting Information), and established the coupling constants (^{3}J values) as 15.3 Hz, 14.8 Hz and 11.2 Hz, respectively. Just prior to biological and chemical investigations, the methyl ester in **13** was subjected to a lenient saponification.[17] LC/MS-MS data confirmed the full conversion of **13** to the chemically labile epoxy acid **9** while UV-experiments confirmed the conservation of the *E,E,Z*-triene. All efforts to obtain NMR-data of epoxy-acid **9** failed due to decomposition. Based on the short half-life time of such compounds,[14b, 16-17, 20] this is not unexpected.



Scheme 2. Synthesis of $ePD_{n\text{-}3\ DPA}$ (**9**).

To obtain further evidence for the physical properties of **9** we assessed the ultraviolet absorbance profile of the synthetic material. This gave data characteristic of a triene chromophore that is allylic to an auxochrome with λ_{max} (MeOH) = 280 nm with shoulders at 260 nm and 293 nm. Addition of acidified water (pH = 3.0) led to a rapid shift in the λ_{max} value from 280 nm to 270 nm, which is consistent with the formation of a conjugated *E,E,E*-triene system (Figure 3A, B). We next assessed the product profile from the hydrolysis experiment of **9** using multiple reaction monitoring (MRM) in liquid chromatography tandem mass spectrometry. This gave four main products with a parent ion of *m/z* 361 that is characteristic of a n-3 docosapentaenoic acid backbone carrying two hydroxyl-groups, consistent with the non-enzymatic hydrolysis of **9** to two pairs of isomers of **5**, see Figure 3C and Scheme 3. Assessment of MS-MS fragmentation spectra for products I and II gave essentially identical spectra that were consistent with that of 10,17(*S*)-dihydroxydocosa-

7*Z*,11*E*,13*E*,15*E*,19*Z*-pentaenoic acid, where water is added at the most activated carbon atom of a carbocation specie (Figure 3D and Scheme 3). Assessment of MS-MS spectra for products under peaks III and IV gave fragmentation patterns that were essentially identical and consistent with products containing a vicinal diol corresponding to 16,17(*S*)-dihydroxydocosa-7*Z*,10*Z*,12*E*,14*E*,19*Z*-pentaenoic acid (Figure 3E and Scheme 3). Of note, in these incubations we did not identify the presence of **5**. This suggests that the aqueous hydrolysis of this product is analogous to that of other allylic epoxides including LTA_4 and 13(*S*),14(*S*)-eMaR1.[16, 21] In addition, these results also indicate that the conversion of **9** to the SPM **5** is regulated by an epoxide hydrolase enzyme. Moreover, a *E*,*E*,*Z*-triene is present in **5**, while the non-enzymatic hydrolysis at C-10 in **9** results in a thermodynamically more stable *E*,*E*,*E*-triene.

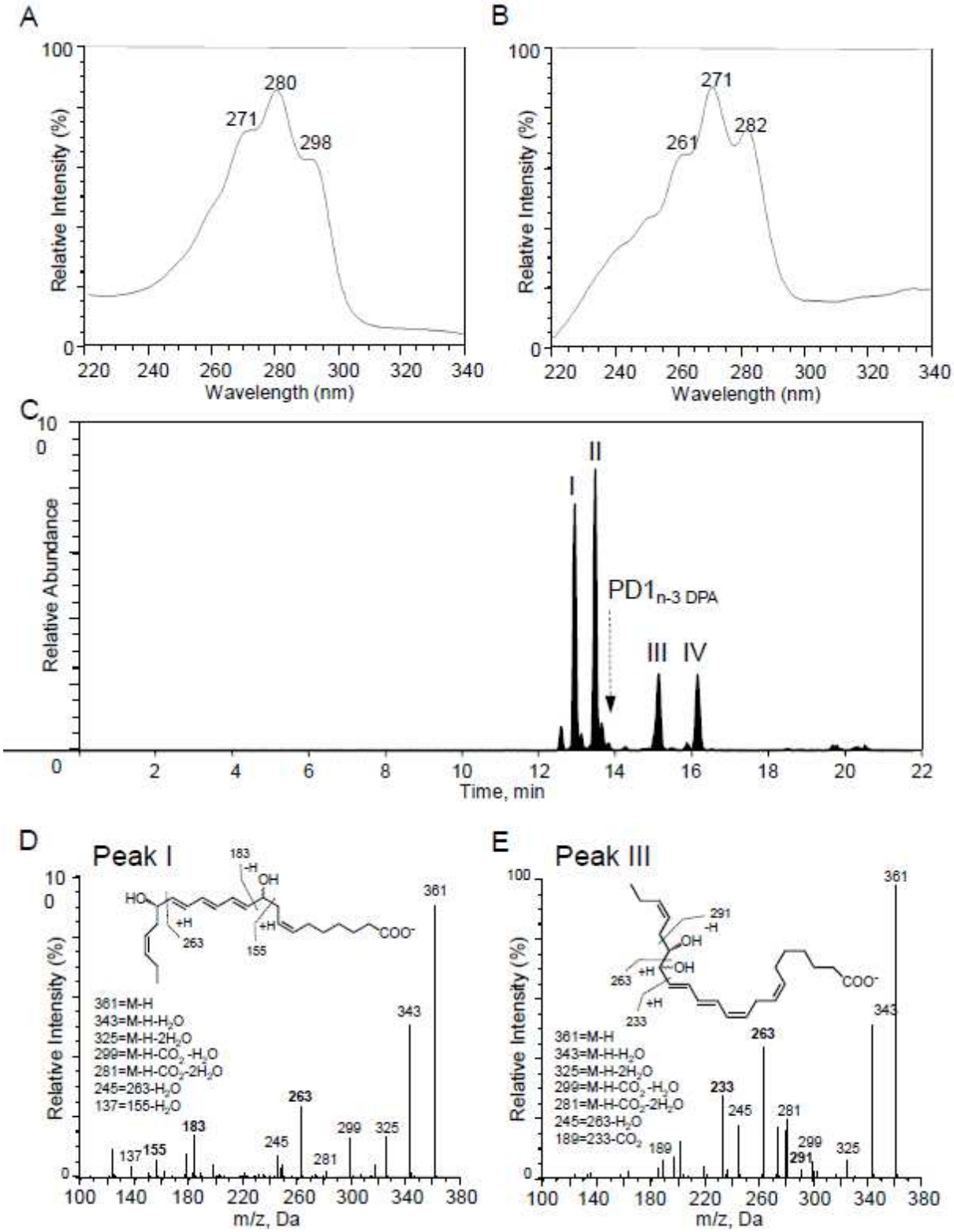
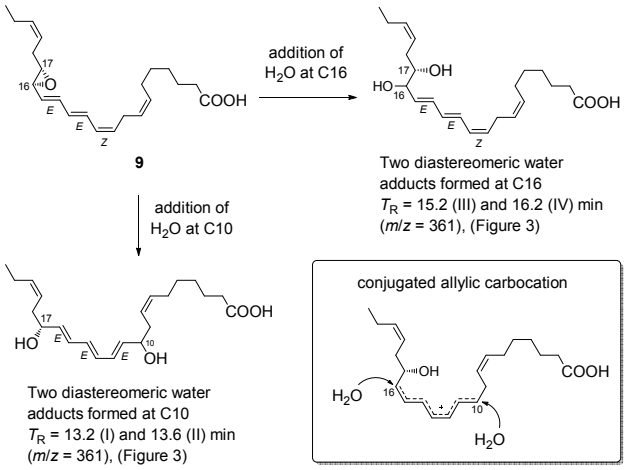


Figure 3. The epoxy acid **9** is rapidly hydrolysed in aqueous conditions. Synthetic **9** (1 ng/1 µL) was placed in double distilled water acidified to pH = 3.0, products were extracted and profiled using lipid mediator profiling. UV chromophore of (A) **9** and (B) non-enzymatic hydrolysis products of **9**. (C) MRM chromatogram of products *m/z* 361 > 263. Arrow denotes retention time of synthetic and authentic **5**. (D, E) MS-MS spectra employed for identification of products under peaks I (D) and III (E). Results are representative of n = 3 incubations.



Scheme 3. Non-enzymatic hydrolysis of synthetic **9** at pH = 3.0.

Human 15-lipoxygenase produces ePD$_{n\text{-}3\ DPA}$

Having determined the physical properties of **9** at pH 3.0, we next determined whether human 15-lipoxygenase was the enzyme responsible for the formation of this intermediate. For this purpose we incubated human recombinant 15-LOX type 1 with n-3 DPA and assessed product profile using LC/MS-MS. In these incubations we identified five main peaks (Figure 4A). Peak I and II gave retention times (T_R) of 13.2 min and 13.6 min and essentially identical MS-MS fragmentation spectra that corresponded to two isomers of 10,17*S*-dihydroxy-7*Z*,11*E*,13*E*,15*E*,19*Z*-docosapentanenoic acid, see Figure 4B, and Scheme 4. The lipoxygenation product 10(*S*),17(*S*)-dihydroxy-7*Z*,11*E*,13*Z*,15*E*,19*Z*-docosapentanenoic acid (**10**) was detected at 13.7 min and with MS-MS fragmentation spectra in accord with literature.[11a] Peaks III and IV gave T_R of 15.2 min and 16.2 min and MS-MS spectra that were consistent with a vicinal diol structure of two isomers, namely 16,17(*S*)-dihydroxy-7*Z*,10*Z*,12*E*,14*E*,19*Z*-docosapentanenoic acid; products that are consistent with the aqueous hydrolysis of an allylic epoxide (Figure 4C and Scheme 4). These observations render support for the formation of **9** from n-3 DPA.

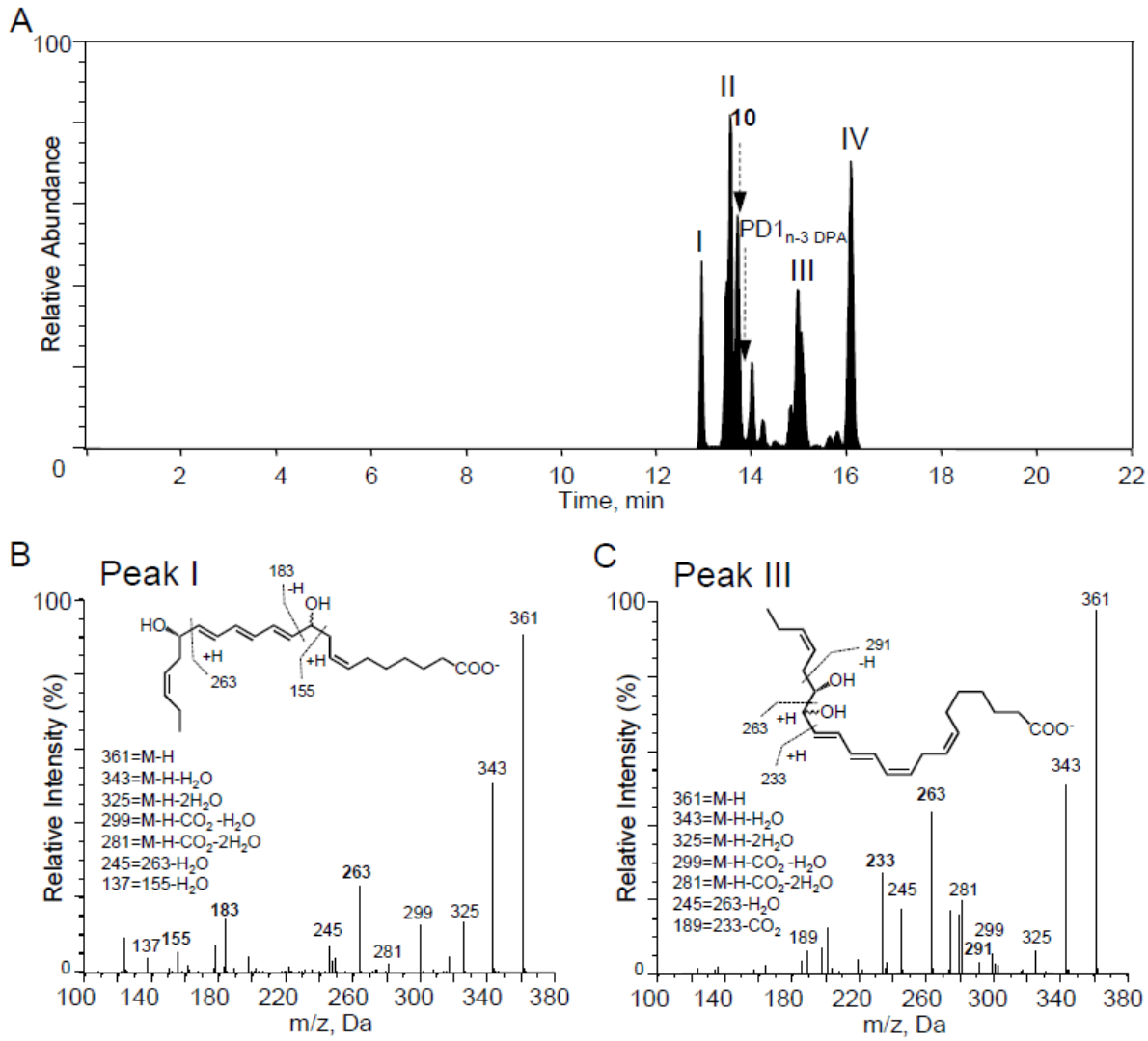
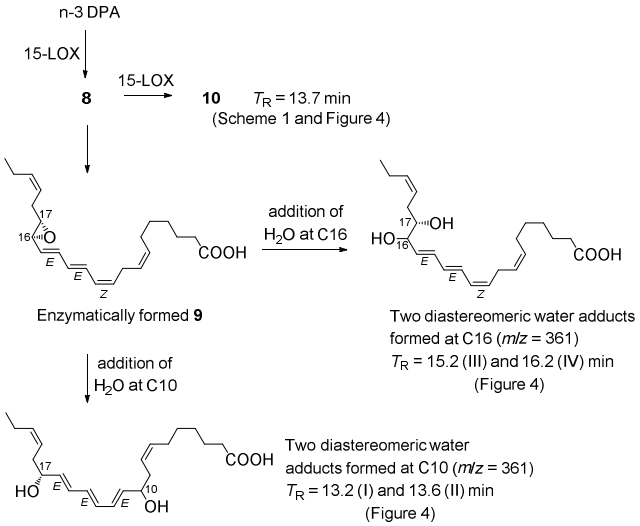


Figure 4. Product profile of human 15-LOX supports the formation of **9** from n-3 DPA. Human 15-LOX was incubated with n-3 DPA (Tris Buffer, pH = 8.0). Incubations were quenched with ice-cold methanol and products profiled using LC/MS-MS-based lipid mediator (LM) profiling. (A) MRM chromatogram of products *m/z* 361 > 263. Arrow denotes retention time of synthetic and authentic **5**. (B, C) MS-MS spectra employed for identification of products under peaks (B) I and (C) III. Results are representative of n = 3 incubations.



Scheme 4. Outline of 15-LOX mediated product formation investigated.

ePD$_{n\text{-}3\ DPA}$ is converted to PD1$_{n\text{-}3\ DPA}$ by human neutrophils

We next tested whether **9** was converted to **5** in human neutrophils (Figure 5A-5D). For this purpose we incubated human peripheral blood neutrophils with synthetic **9** and assessed the product profile using LC/MS-MS based profiling. Searching for the ion pair with an *m/z* 361 > 263 using multiple reaction monitoring we obtained a sharp peak that eluted with a T_R of 13.8 min in reverse phase liquid chromatography (Figure 5A). The MS-MS fragmentation spectrum for the product under this peak gave ions *m/z* 361 = M-H, *m/z* 343 = M-H-H_2O-CO_2, *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* 245 = 263-H_2O, *m/z* 219 = 263-CO_2, *m/z* 201 = 263-H_2O-CO_2 and *m/z* 138 = 183-CO_2, consistent with data reported earlier for both authentic[11a] and synthetic **5**[13] (Figure 5B). When **9** was either incubated with neutrophils that had been previously kept at 100 ^oC for 1 h (denaturated cells) or in phosphate buffered saline, we found a significantly lower concentrations of **5** (Figure 5C, D). These results demonstrate that synthetic **9** is indeed the biosynthetic intermediate for **5** and provide further evidence supporting the role of an epoxide hydrolase in catalyzing this biosynthetic step. Overall, the observations presented are in accordance with the reaction pathway reported by Corey and Mehrotra[21a] and those observed for enzyme mediated mechanisms involved in the biosynthesis of other pro-resolving mediators.[21b]

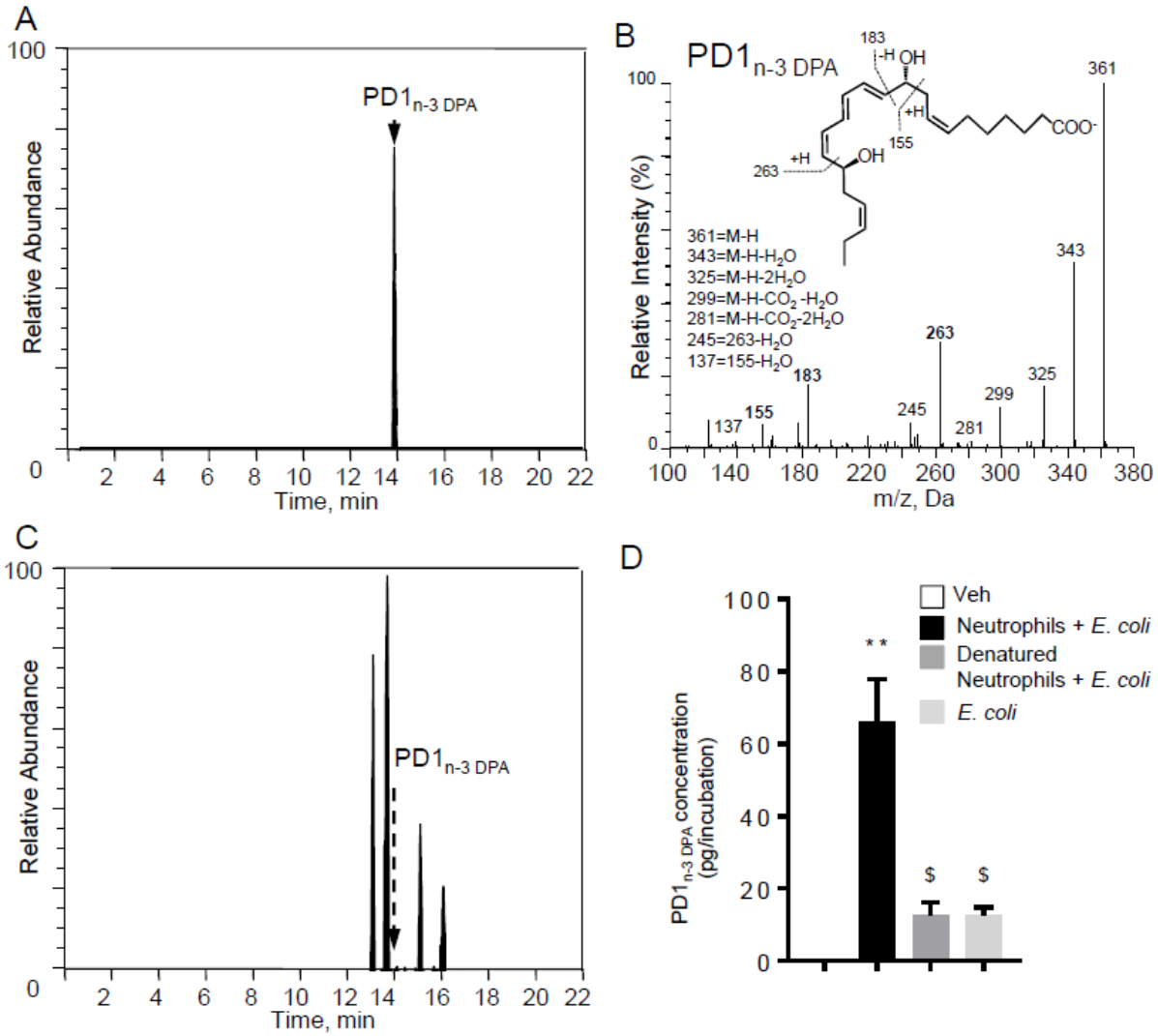


Figure 5. Epoxy acid **9** is converted to **5** by human neutrophils. Synthetic epoxy acid **9** (10 nM) was incubated with human primary neutrophils (5 x 10^6 cells), human neutrophils previously (5 x 10^6 cells) incubated at 100 °C for 1 h (denatured cells), or PBS. To these incubations *E. coli* (2.5 x 10^8 CFU/mL) were added and cells incubated for 15 min, 37 °C. Veh denotes solution containing 0.1% EtOH in PBS (A) MRM chromatogram of **5** *m/z* 361 > 155. (B) MS-MS spectrum employed for identification of **5**. (C) MRM chromatogram of products *m/z* 361 > 263. (D) Concentrations of **5** in incubations. Results are representative of n = 4 incubations. ** $P < 0.01$ vs Veh incubations. $ < 0.05 vs neutrophils + *E. coli* incubations.

ePD$_{n\text{-}3\ DPA}$ inhibits human neutrophils LTB$_4$ production

Given that allylic epoxides including LTA_4[22] and 13(*S*),14(*S*)-eMaR1[16] are known to regulate the activity of lipid mediator biosynthetic enzymes *via* suicide inactivation we next questioned whether **9** also shared this biological activity. Here we investigated whether synthetic **9** regulated LTB_4 biosynthesis in human neutrophils, a biosynthetic step that is catalyzed by leukotriene A_4 hydrolase. Synthetic **9** dose-dependently reduced LTB_4 concentrations in human peripheral blood neutrophil incubations with *E. coli* at

concentrations as low as 0.1 nM. This reduction in LTB_4 production was equivalent to that observed with equimolar concentrations of the pro-resolving mediator **5** (Figure 6). Therefore these results indicate that the epoxide also carries anti-inflammatory actions and regulates human leukocyte responses.

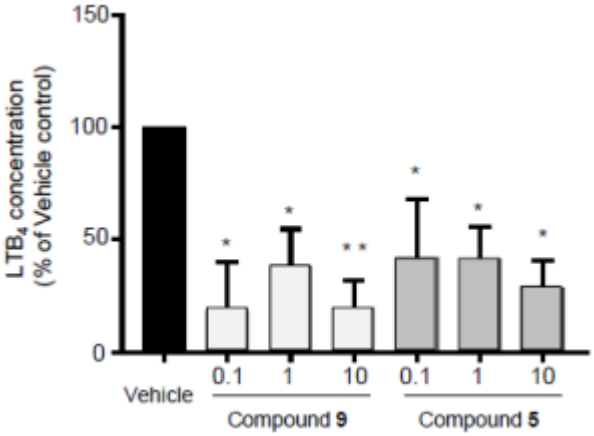


Figure 6. The epoxy acid **9** and **5** inhibit human neutrophil LTB_4 production. Human neutrophils (5 x 10^6 cells) were incubated with either **9** or **5** at 0.1, 1 or 10 nM; 37 °C; PBS (pH = 7.45) for 5 min, then with *E. coli* (2.5 x 10^8 CFU/mL) for 30 min. Incubations were quenched and LTB_4 concentrations determined using LM profiling. Results are percentage of vehicle incubations. $n = 4$, $^*p < 0.05$, ** $p < 0.01$ *vs* vehicle.

Conclusions

To summarize, $ePD_{n\text{-}3\ DPA}$ (**9**) has been prepared via a stereoselective total synthesis, which allowed the exact structural assignment of **9**. Moreover, it has now been established that the biosynthesis of $PD1_{n\text{-}3\ DPA}$ in neutrophils occurs with **9** as an intermediate as outlined in Scheme 1. In addition, we have shown that 15-LOX is involved in the conversion of n-3 DPA into **9**. Moreover, the formation of **5** in human leukocytes and the absence of formation of **5** in acidic aqueous conditions as well as the diminished biosynthetic formation of **5** using denatured neutrophils, supports the presence of a hydrolytic enzyme involved in the conversion of **9** to **5**. Finally, we found that the epoxy acid **9** regulates the formation of the potent neutrophil chemoattractant LTB_4, with equal potencies to that obtained with $PD1_{n\text{-}3\ DPA}$. The inhibition of LTB_4 formation by synthetic inhibitors[23] or by SPM biosynthesis is of interest in drug discovery research,[24] as recently reported for the SPM $PD1_{n\text{-}3\ DPA}$.[25]

Experimental Section

Synthesis of methyl (7*Z*,10*Z*,12*E*,14*E*)-15-((2*S*,3*S*)-3-((*Z*)-pent-2-en-1-yl)oxiran-2-yl)pentadeca-7,10,12,14-tetraenoate (13)

The Wittig-salt **12**[19] (86 mg, 0.15 mmol, 1.3 equiv.) was dissolved in THF (1.7 mL) and HMPA (0.26 mL) was cooled to −78 °C. NaHMDS (0.60 M in toluene, 0.25 mL, 0.15 mmol, 1.3 equiv.) was added dropwise, and the reaction mixture was stirred for 30 min, giving an orange, completely homogeneous solution. Aldehyde **11**[17] (22 mg, 0.11 mmol, 1.0 equiv.) was azeotroped twice with 2-methyltetrahydrofuran, dissolved in THF (0.85 mL), cooled to −78 °C, and added dropwise to the reaction flask *via* cannula. After 1 h, the flask was rapidly warmed to −20 °C for a few minutes and then quenched by the addition of phosphate buffer (pH = 7, 3.5 mL). The phases were separated, and the aqueous phase was extracted with Et_2O (3 × 5.0 mL). The combined organic layers were dried (Na_2SO_4), filtrated, and concentrated *in vacuo*. The crude material was loaded onto a small column of silica gel that was prepared as a slurry using 3% Et_3N, 20% Et_2O in heptane, and the column was eluted with 25% Et_2O in heptane to provide the epoxy methyl ester **13** in 58% yield (24 mg) as a colorless oil: $[\alpha]_D^{20}$ = −38 (c = 0.20, $CHCl_3$); UV (hexane) λ_{max} 270, 281, 292; ^{1}H NMR (600 MHz, Benzene-d_6) δ 6.54 (dd, *J* = 14.7, 11.4 Hz, 1H), 6.39 (dd, *J* = 15.3, 10.8 Hz, 1H), 6.11 (dd, *J* = 14.8, 10.9 Hz, 1H), 6.04 (t, *J* = 11.2 Hz, 1H), 5.51 – 5.31 (m, 6H), 3.36 (s, 3H), 3.06 (dd, *J* = 7.8, 1.8 Hz, 1H), 2.91 (t, *J* = 7.1 Hz, 2H), 2.71 (td, *J* = 5.2, 2.0 Hz, 1H), 2.24 (dq, *J* = 13.8, 6.4 Hz, 1H), 2.20 – 2.14 (m, 1H), 2.09 (t, *J* = 7.4 Hz, 2H), 1.94 (dq, *J* = 23.1, 8.4, 7.5 Hz, 4H), 1.53 (p, *J* = 7.4 Hz, 2H), 1.25 – 1.13 (m, 4H), 0.88 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (151 MHz, C_6D6) δ 173.3, 134.7, 134.1, 132.3, 131.4, 131.4, 1308, 129.0, 128.9, 127.6, 123.2, 60.3, 57.7, 51.0, 34.1, 30.1, 29.6, 29.0, 27.4, 26.7, 25.2, 21.0, 14.4; HRMS (TOF ES$^+$): Exact mass calculated for $C_{23}H_{34}O_3Na$ $[M+Na]^+$: 381.2400, found 381.2400. TLC (heptane/Et_2O, 74:26 CAM stain): R_f = 0.21.

(7*Z*,10*Z*,12*E*,14*E*)-15-((2*S*,3*S*)-3-((*Z*)-pent-2-en-1-yl)oxiran-2-yl)pentadeca-7,10,12,14-tetraenoic acid (ePD$_{n\text{-}3\ DPA}$, 9)

A stock solution of 100 μg of ester **13** in hexane/Et_2O was dried under a gentle stream of nitrogen and then dissolved in 500 μL of THF and cooled to -78 ^{o}C using a dry ice/ isopropanol cooling bath. Then 100 μL of aqueous 1.0 M LiOH solution was slowly added via a Hamilton syringe at -78 ^{o}C. Then additional 100 μL of H_2O was added and the vial was covered with aluminium foil and left stirring for 10 h. The solution above the precipitated lithium salt was gently removed by using a syringe. Next, the THF-solution was dried under a gentle stream of nitrogen before quantification using UV (hexane) λ_{max} 271 (log ε 3.58), 280

(log ε 4.08), 298 (log ε 2.69) nm. After quantification and removal of hexane, **9** was suspended in 50 μL $PBS^{+/+}$ pH = 7.45. The PBS solution was kept on solid dry ice in a closed container prior to use, and was used for incubation experiments. The structure of the chemical labile free acid **9** was determined indirectly by using UV and LC/MS-MS experiments (Figure 3A, Figure 3C and Figures 4-5).

(7*Z*,10*R*,11*E*,13*E*,15*Z*,17*S*,19*Z*)-10,17-dihydroxydocosa-7,11,13,15,19-pentaenoic acid ($PD1_{n\text{-}3\ DPA}$, 5)

The synthesis of **5** has been reported earlier.[13]

Lipid mediator profiling

$ePD_{n\text{-}3\ DPA}$ (**9**) (10 ng) was placed in double-distilled water (pH = 3.0), incubated at room temperature for five minutes, methanol containing 500 pg d_5-LTB_4 (Cayman Chemicals) was added, and the products profiled using LC/MS-MS based lipid mediator as detailed below. In separated experiments cell and recombinant enzyme incubations were quenched using 2 volumes of ice-cold methanol d_5-LTB_4 (500 pg Cayman Chemicals) and lipid mediators were extracted using C-18 based Solid Phase Extraction as recently reported.[26] Methyl formate fractions were brought to dryness using a TurboVap LP (Biotage) and products suspended in water-methanol (80:20 vol:vol) for Liquid Chromatography-tandem mass spectrometry (LC-MS/MS) based profiling. Here a Shimadzu LC-20AD HPLC and a Shimadzu SIL- 20AC autoinjector (Shimadzu, Kyoto, Japan), paired with a QTrap 5500 (ABSciex, Warrington, UK) were utilised and operated as described.[26] To monitor each lipid mediator and deuterium labelled internal standard, a multiple reaction monitoring (MRM) method was developed using parent ions and characteristic diagnostic ion fragments.[26] This was coupled to an Information Dependent Acquisition and an Enhanced Product Ion scan. Identification criteria included matching retention time (T_R) to synthetic standards and at least six diagnostic ions in the MS-MS spectrum for each molecule. Calibration curves were obtained for each molecule using authentic compound mixtures and deuterium labelled lipid mediator at 0.78, 1.56, 3.12, 6.25, 12.5, 25, 50, 100, and 200 pg. Linear calibration curves were obtained for each lipid mediator, which gave r^2 values of 0.98-0.99.

Human neutrophil incubations

Human peripheral blood neutrophils were isolated from healthy volunteers using density-gradient Ficoll-Histopaque isolation. Volunteers gave written consent in accordance with a

Queen Mary Research Ethics Committee (QMREC 2014:61) and the Helsinki declaration. Isolated neutrophils were suspended in $PBS^{+/+}$ containing 10% BSA and incubated for 10 min (37 °C, pH = 7.45) prior to the addition of the indicated concentrations of **9** and *E. coli* (2.5×10^8 cells/mL). Incubations were quenched using 2 volumes ice-cold methanol containing, d_5-LTB_4 was added to facilitate quantification and identification, and products were extracted using C18 columns and quantified by LC-MS-MS metabololipidomics as detailed above. In selected experiments neutrophils were incubated at 100 °C for 1 h prior to addition of $ePD_{n\text{-}3\ DPA}$ (**9**) and *E. coli* and incubations conducted as detailed above.

Aqueous hydrolysis of $ePD_{n\text{-}3\ DPA}$ (9)

The UV absorbance spectrum of **9** was measured using an Agilent Technology Cary 8485 UV-Vis spectrophotometer. Acidified water (pH = 3.0) was added, and after five minutes at room temperature, the UV absorbance spectrum was measured.

Human 15-LOX incubations

Human recombinant 15-LOX (Novus Biologicals) was incubated with n-3 DPA (1 µM) in Tris buffer (pH = 8.0) at room temperature for 15 min. Products were quenched and extracted as detailed in the lipid mediator profiling section above.

Acknowledgment

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Notes and references

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