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Total Synthesis of the Lipid Mediator PD1_{n-3} DPA: Configurational Assignments and Anti-inflammatory and Pro-resolving Actions

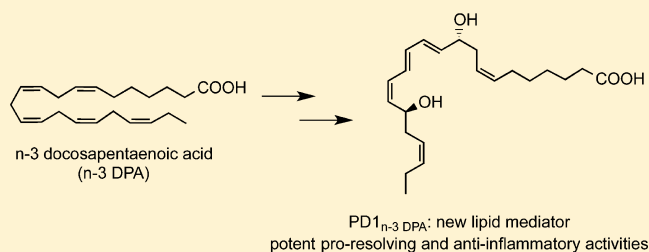
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S Supporting Information

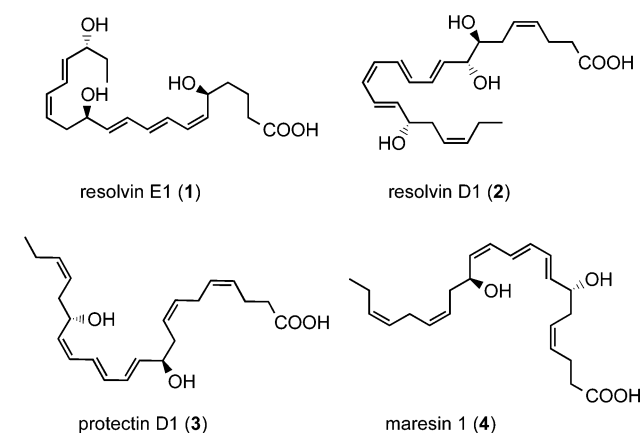
ABSTRACT: The polyunsaturated lipid mediator PD1_{n-3} DPA (**5**) was recently isolated from self-resolving inflammatory exudates of **5** and human macrophages. Herein, the first total synthesis of PD1_{n-3} DPA (**5**) is reported in 10 steps and 9% overall yield. These efforts, together with NMR data of its methyl ester **6**, confirmed the structure of **5** to be (7*Z*,10*R*,11*E*,13*E*,15*Z*,17*S*,19*Z*)-10,17-dihydroxydocosa-7,11,13,15,19-pentaenoic acid. The proposed biosynthetic pathway, with the involvement of an epoxide intermediate, was supported by results from trapping experiments. In addition, LC-MS/MS data of the free acid **5**, obtained from hydrolysis of the synthetic methyl ester **6**, matched data for the endogenously produced biological material. The natural product PD1_{n-3} DPA (**5**) demonstrated potent anti-inflammatory properties together with pro-resolving actions stimulating human macrophage phagocytosis and efferocytosis. These results contribute new knowledge on the n-3 DPA structure–function of the growing numbers of specialized pro-resolving lipid mediators and pathways.



Resolution of inflammation is necessary to re-establish homeostasis after injury or infection.^{1–3} Excessive inflammatory responses that fail to undergo resolution may lead to chronic inflammation associated with many diseases, such as asthma, atherosclerosis, autoimmune diseases, cancer, and neuropathological disorders, including Alzheimer's and Parkinson's diseases.^{1,2} Hence, over the last century, inflammation has been the topic of numerous studies at the molecular and cellular level.³ As of today, several chemical mediators have been identified that can initiate, modulate, and reduce acute inflammation and stimulate resolution.⁴ Continued efforts have established that the return to homeostasis, catabasis,⁵ is mediated by active biosynthesis and termination programs orchestrated by novel families of natural products coined specialized pro-resolving mediators (SPMs).⁶ The SPMs are derived from polyunsaturated fatty acids (PUFAs) during the resolution phase of acute inflammation.^{6,7} These efforts have provided the fundamentals for the molecular understanding of the resolution of many inflammatory diseases.⁷

Recent studies have identified several novel SPMs biosynthesized from the dietary n-3 PUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These novel di- and trihydroxy-containing PUFA-derived lipid mediators are termed resolvins, protectins, and maresins. Resolvin E1 (**1**) is produced from EPA,⁸ while resolvin D1 (**2**),^{9,10} protectin D1 (PD1, **3**),^{9–12} also known as neuroprotectin D1 (NPD1) when

produced in neural systems,⁹ and maresin 1 (**4**)¹³ are produced from DHA.



These mediators are examples of SPMs structurally elucidated within the past decade showing potent in vivo anti-inflammatory and pro-resolving activities resulting in catabasis.⁵ It is now well established that EPA and DHA are substrates for the enzymatic formation of the aforementioned SPMs. The naturally occurring compounds **1–4** have been the

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subject of many pharmacological studies for the development of potential new anti-inflammatory drugs,^{14,15} and some have already been the subject of clinical trials.¹⁵ These autacoids exhibit stereochemically selective modes of actions, reflecting their routes of biosynthesis.¹⁵ In light of this, we became interested in investigations using other n-3 PUFAs than EPA and DHA as potential substrates for the endogenous formation of novel SPMs. In humans, genome-wide association studies demonstrate that elevation in circulating levels of n-3 docosapentaenoic acid (n-3 DPA), or (7*Z*,10*Z*,13*Z*,16*Z*,19*Z*)-docosapentaenoic acid, with a concomitant decrease in DHA levels, are associated with single nucleotide polymorphisms in the gene encoding for the fatty acid elongase 2 (ELOVL2).¹⁶ The levels of circulating n-3 DPA do not appear to be directly associated with dietary intake of PUFAs.^{17,18} Recently Dalli et al. reported that n-3 DPA was converted to novel SPMs by both human and murine leukocytes.¹⁹ One of the novel products identified was assigned the structure (7*Z*,11*E*,13*E*,15*Z*,17*S*,19*Z*)-10,17-dihydroxydocosa-7,11,13,15,19-pentaenoic acid and denoted PD1_{n-3 DPA} (**5**), given its relation to PD1 (**3**)²⁰ (Figure 1).

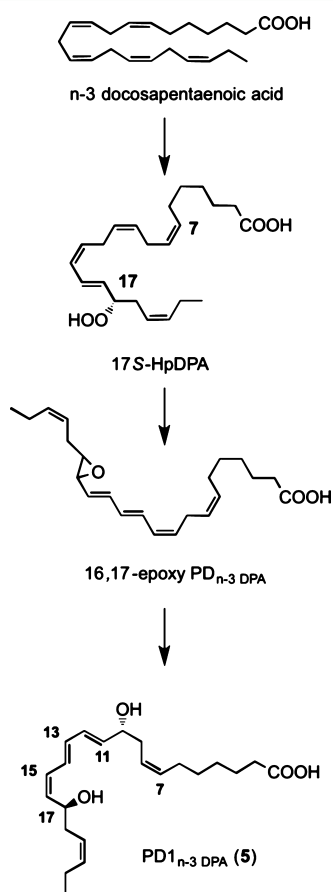


Figure 1. Proposed biosynthesis of PD1_{n-3 DPA} (**5**).

The only apparent structural difference between DHA and n-3 DPA is the absence of a *cis*-double bond at the C-4 position. This difference is proposed to confer unique biophysical properties relevant for biological functions.²¹ With the aim of providing multimilligram quantities for biological studies, the synthesis of n-3 DPA was achieved.²²

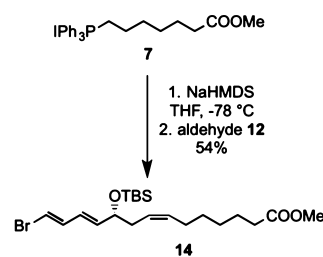
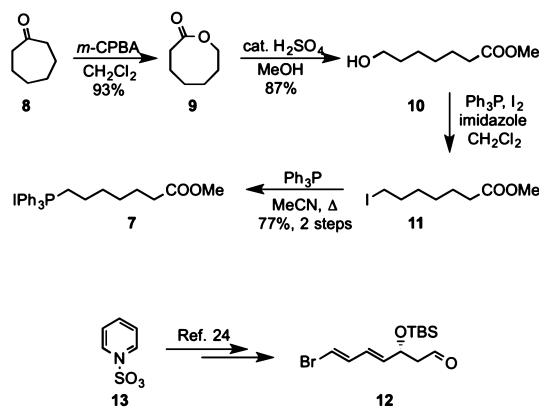
The initial structural assignment of the n-3 DPA-derived compound PD1_{n-3 DPA} (**5**) was based on biosynthetic results

with human polymorphonuclear leukocytes (PMN) followed by chromatographic purification employing LC-MS/MS fragmentation. Moreover, the natural product **5** exhibited identical chromatographic properties with RvD5_{n-3 DPA} or (7*S*,8*E*,10*Z*,13*Z*,15*E*,17*S*,19*Z*)-7,17-dihydroxydocosa-8,10,13,15,19-pentaenoic acid, produced under the same experimental conditions.¹⁹ Therefore, the full stereochemical assignment of the double-bond geometry in the conjugated triene system as well as the absolute configuration of the C-10 group remained to be established. Also, the assignment of the *S*-configuration on carbon atom 17 was based on biosynthetic considerations. Hence, total synthesis became necessary for establishing the complete configurational assignment of **5**, as has been necessary for the novel SPMs of interest, but also due to the pico- to nanogram amounts produced *in vivo*.^{15,19} In addition, the biological actions of **5** remained to be confirmed. Herein, we report the synthesis and the elucidation of the absolute configuration of SPM **5** based on matching data from both LC-MS/MS and GC/MS analyses of synthetic and endogenous materials, as well as 1D- and 2D-NMR spectroscopic data of its methyl ester **6**. The novel bioactions of **5** are also reported.

RESULTS AND DISCUSSION

The synthesis of PD1_{n-3 DPA} (**5**) commenced with the preparation of the Wittig salt **7** in four steps from cycloheptanone (**8**). First, a Baeyer–Villiger oxidation²³ of **8** yielded lactone **9**, which was reacted with MeOH and catalytic amounts of H₂SO₄ to produce methyl-7-hydroxyheptanoate (**10**). The alcohol functionality in **10** was converted into the corresponding iodide **11**. Reacting triphenylphosphine with **11** afforded the desired Wittig salt **7** in 62% yield from **8** (Scheme 1). Then (3*R*,4*E*,6*E*)-7-bromo-3-((*tert*-butyldimethylsilyloxy)hepta-4,6-dienal (**12**) was prepared as previously reported from salt **13**.²⁴ Aldehyde **12** was reacted with the ylide of **7**, the latter obtained

Scheme 1. Synthesis of Wittig Salt **7** and Intermediate **14**



after reaction with NaHMDS in THF at $-78\text{ }^{\circ}\text{C}$, in a *Z*-selective Wittig reaction. After purification using silica gel chromatography, pure vinylic bromide ester **14** was obtained in 54% yield (Scheme 1). The stereoisomeric purity of **14** was determined by HPLC and ^1H NMR analyses (Supporting Information).

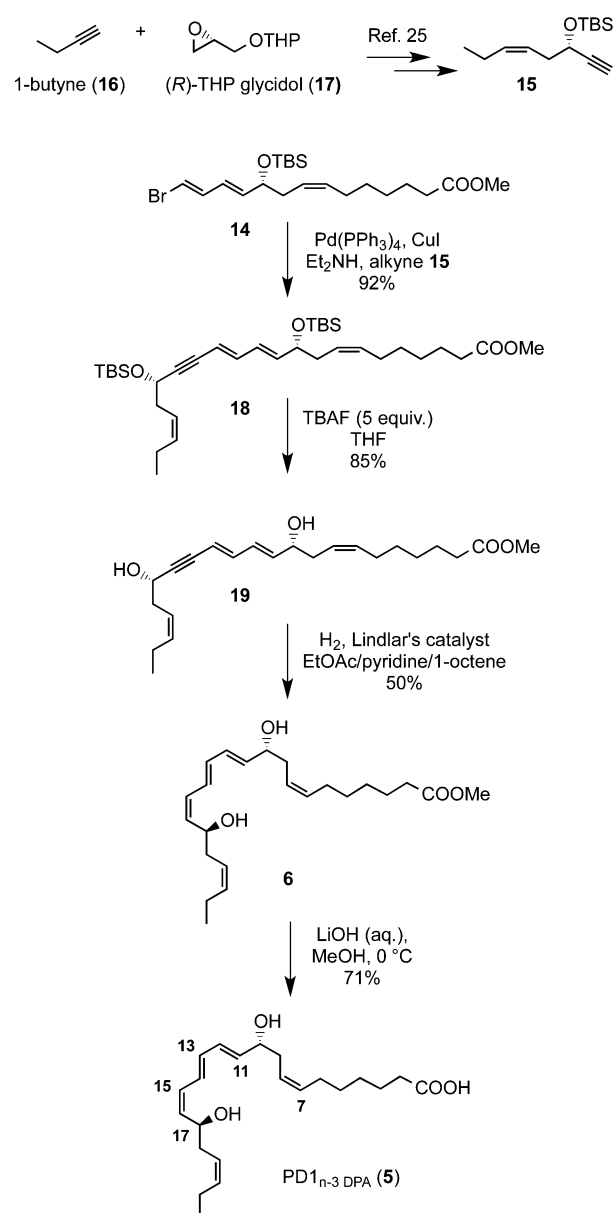
The alkyne **15** was synthesized essentially as previously reported from 1-butyne (**16**) and (*R*)-THP glycidol (**17**).²⁵ Then vinylic bromide ester **14** and alkyne **15** were reacted in a Sonogashira reaction²⁶ at ambient temperature in the presence of catalytic $\text{Pd}(\text{PPh}_3)_4$ and CuI with diethyl amine as solvent. This afforded the bis-TBS-protected methyl ester **18** in 92% yield. Deprotection of the two TBS groups in **18** with five equivalents of TBAF in THF at $0\text{ }^{\circ}\text{C}$ gave an 85% yield of the diol **19**.²⁷ The internal alkyne in **19** was reduced to the *Z,E,E,Z,Z*-pentaene ester **6** in 50% yield using a modified Lindlar hydrogenation reaction²⁸ with high stereoselectivity, as only one diastereomer was detected by HPLC and ^1H NMR analyses. Finally, hydrolysis of the methyl ester **6** at $0\text{ }^{\circ}\text{C}$ with dilute aqueous LiOH in MeOH followed by mild acidic workup with aqueous NaH_2PO_4 ²⁹ resulted in a 71% yield of $\text{PD1}_{n-3}\text{ DPA}$ (**5**) after purification by column chromatography (Scheme 2).

The assignment of the *Z*- or *E*-geometry for each of the double bonds was achieved by two-dimensional NMR spectroscopy, with $\text{MeOH-}d_4$ as the solvent and internal standard. These experiments revealed the connectivity between adjacent olefinic hydrogens (H-7/H-8, H-11/H-16, and H-19/H-20), which in connection with the data from the HMBC spectra permitted the assignment of all olefinic hydrogens. In particular, the signals at 5.74 (dd, 1H, $J = 14.4\text{ Hz}$) and 6.07 ppm (t, 1H, $J = 11.0\text{ Hz}$) were diagnostic for an *E*- and *Z*-double bond, respectively. The COSY spectrum was used for assigning vicinal signals for both of the two isolated *Z*-olefins and the *E,E,Z*-triene moiety. Moreover, two signals from the hydrogen atoms attached to the carbinol carbon atoms were observed as expected with signals at 4.12 (m, 1H) and 4.56 ppm (dt, 1H, $J = 8.9, 6.8\text{ Hz}$). The HSQC spectrum was used for assigning the signals from the methylene carbons at 36.36 and 36.38 ppm, next to the C-19/C-20 and the C-7/C-8 *Z*-double bonds, respectively. The data from the ^1H and ^{13}C NMR spectra, in combination with the COSY and the HMBC spectra, allowed the structural assignment of the rest of the molecule (Table 1).

UV spectra for both synthetic and endogenously produced $\text{PD1}_{n-3}\text{ DPA}$ (**5**) were compared. For synthetic **5**, $\lambda_{\text{max}}^{\text{MeOH}}$ absorbances were observed at 262, 271, and 282 nm; all in excellent agreement with the endogenous product¹⁸ (261, 271, 282 nm, $\lambda_{\text{max}}^{\text{MeOH}}$), as well as with literature values.³⁰ Synthetic **5** was treated with excess diazomethane followed by *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) to produce the bis-TMS ether of methyl ester **6**, which was subjected to GC/MS (Supporting Information, Figure S-25). The following characteristic m/z values were observed: 520 = M , 505 = $M - \text{CH}_3$, 489 = $M - \text{OCH}_3$, 430 = $M - \text{OTMS}$, 340 = $M - 2 \times \text{OTMS}$, and 73 = TMS.

Experiments supporting the proposed biosynthetic pathway in Figure 2 were then performed. Soybean lipoxygenase was incubated with *n*-3 DPA in borate buffer (pH = 8.0) at ambient temperature essentially as described.⁹ Excess acidic MeOH was added to quench the reaction and promote opening of the epoxide. Then the product mixture was assessed by lipid mediator metabololipidomics.^{31,32} From the MS/MS fragmentation patterns of the formed products, four structures were assigned (Figure S-26). These results render support for the

Scheme 2. Final Steps of the Synthesis of $\text{PD1}_{n-3}\text{ DPA}$ (**5**)



proposed biosynthetic pathway¹⁹ as depicted in Figure 2, involving an acid-catalyzed ring-opening of the 16,17-epoxy $\text{PD1}_{n-3}\text{ DPA}$. In addition, these observations are in accordance with the reaction pathway reported by Corey and Mehrotra³³ as well as enzyme-catalyzed mechanisms in the biosynthesis of other pro-resolving mediators.³⁴

In order to determine whether synthetic **5** matched endogenous $\text{PD1}_{n-3}\text{ DPA}$ (**5**), authentic **5** from murine self-resolving exudates as well as human macrophages was employed.¹⁹ Figure 2A shows human macrophage $\text{PD1}_{n-3}\text{ DPA}$ (**5**) from *n*-3 DPA with a retention time (t_R) of 12.4 min, together with its natural isomers. Figure 2B depicts endogenous $\text{PD1}_{n-3}\text{ DPA}$ (**5**) from mouse peritoneal exudates that also displayed $t_R = 12.4\text{ min}$. This is shown together with its natural isomers produced in mouse peritoneal exudates. The chromatographic behavior of synthetic **5** ($t_R = 12.4\text{ min}$), obtained from hydrolysis of synthetic methyl ester **6**, is shown in Figure 2C. Figure 2D reports the co-injection of synthetic and endogenously obtained material added at essentially equal

Table 1. Compilation of ^1H and ^{13}C NMR Data of the Methyl Ester 6^a

position	δ_{C} , mult. ^b	δ_{H} , mult. (J in Hz) ^c	HMBC ^d	COSY
1	175.9, C			
2	34.8, CH ₂	2.31, m	1, 3, 4	3
3	25.9, CH ₂	1.60, quint (7.3)	1, 2, 4, 5	2, 4
4	29.8, CH ₂	1.33, m	2, 3, 5, 6	3, 5
5	30.3, CH ₂	1.37, m	3, 4, 6, 7	4, 6
6	28.2, CH ₂	2.04, m	4, 5, 7, 8	5, 7
7	132.8, CH	5.47, m	5, 6, 9	6, 8
8	126.2, CH	5.40, m	6, 9, 10	7, 9
9	36.4, CH ₂	2.26 + 2.32, m	7, 8, 10, 11	8, 10
10	73.1, CH	4.12, m	8, 9, 11, 12	9, 11
11	138.0, CH	5.74, dd (14.4, 6.7)	9, 10, 13	10, 12
12	131.3, CH	6.26, m	10, 11, 13, 14	11, 13
13	134.9, CH	6.24, m	11, 12, 15	12, 14
14	128.9, CH	6.52, dd (13.8, 11.2)	12, 13, 15, 16	13, 15
15	130.5, CH	6.07, t	13, 14, 16, 17	14, 16
16	134.8, CH	5.38, m	14, 18	15, 17
17	68.6, CH	4.56, dt (8.9, 6.8)	15, 16, 18, 19	16, 18
18	36.4, CH ₂	2.36 + 2.21, m	16, 17, 19, 20	17, 19
19	125.3, CH	5.34, m	17, 18, 20, 21	18, 20
20	134.7, CH	5.45, m	18, 19, 21, 22	19, 21
21	21.7, CH ₂	2.07, m	19, 20, 22	20, 22
22	14.6, CH ₃	0.97, t (7.5)	20, 21	21
23	52.0, CH ₃	3.65, s	1	

^aMeOH-*d*₄ was used as solvent. See Figure 1 for atom labeling.

^bMeasured at 125 MHz. ^cMeasured at 500 MHz. ^dHMBC correlations are from proton(s) stated to the indicated carbon(s). The ppm values listed above for δ_{H} were assigned using the center of the COSY and HSQC peak intensities.

amounts. Altogether, Figure 2 demonstrates that synthetic **5** coelutes with endogenously obtained **5**. In addition, the MS/MS spectra for both natural and synthetic **5** were essentially identical (Figure S-24).

Having established that the physical properties of synthetic **5** matched those of endogenous **5**, we next required the confirmation of its anti-inflammatory and pro-resolving actions. Administration of synthetic **5** at a dose as low as 10 ng per mouse significantly reduced neutrophil recruitment during peritonitis, giving a significant reduction in the number of neutrophils recovered from peritoneal exudates following zymosan (1 mg/mouse) challenge determined by light microscopy and flow cytometry (Figure 3A). These actions were comparable to those displayed by the potent DHA-derived pro-resolving mediator protectin D1 (**3**).²⁰ Human macrophage phagocytosis and efferocytosis (clearance of apoptotic neutrophils) are key processes in the resolution of inflammation⁵ and defining actions of SPMs.⁶ The ability of synthetic **5** in this system to stimulate human macrophage phagocytosis and efferocytosis was investigated.¹³ Incubation of synthetic **5** with human macrophages and increasing concentrations of **5** led to an increase in macrophage phagocytosis of both fluorescence-labeled yeast cell wall particles (zymosan) and apoptotic neutrophils. These actions were shared with the DHA-derived protectin D1 (**3**) (Figure 3B). In addition, PD1_{n-3}DPA (**5**) displayed potent efferocytosis effects against apoptotic human neutrophils (Figure 3C). PD1_{n-3}DPA stimulated proresolving actions with macrophages at picomolar concentrations and appeared to give a trend toward a bell-shaped dose–response with these isolated human cells. These

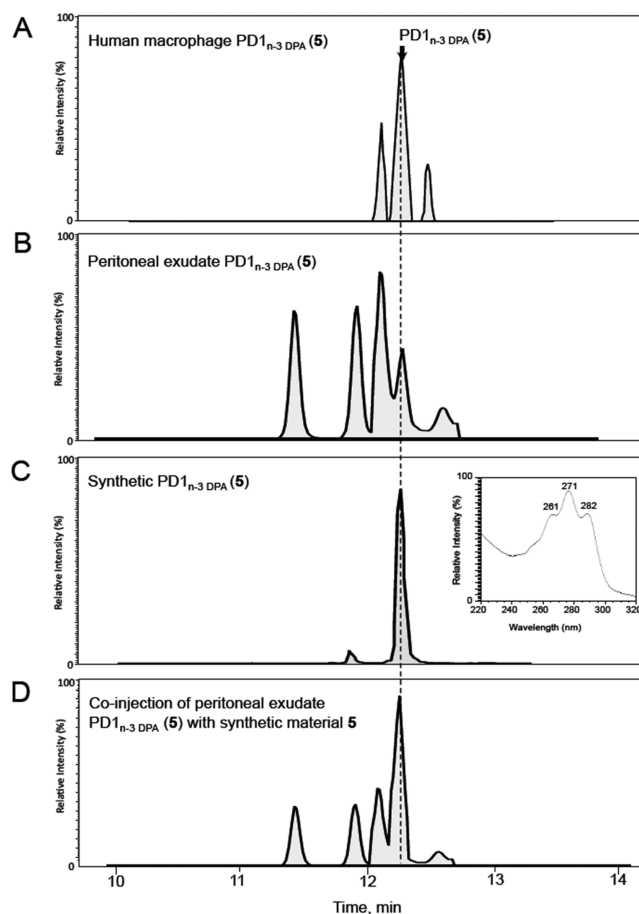


Figure 2. Endogenous PD1_{n-3}DPA (**5**) from human macrophages and resolving inflammatory exudates match synthetic material. MRM chromatograms for selected ion pair *m/z* 361–183 depicting (A) PD1_{n-3}DPA (**5**) from human macrophages (5×10^7 cells/mL) incubated with 0.1 mg of opsonized zymosan and *n*-3 DPA (1 μM , 37 °C, 30 min, DPBS^{+/+}, pH = 7.45). Results are representative of *n* = 3 human macrophage preparations. (B) Endogenous PD1_{n-3}DPA (**5**) obtained from mice injected with zymosan (1 mg/mouse) and exudates collected at 4 h. Results are representative of *n* = 4 mice exudates. (C) Synthetic material (inset: characteristic UV-absorption spectrum, $\lambda_{\text{max}}^{\text{MeOH}} \pm 1$ nm). (D) Co-injection of resolving exudate endogenous PD1_{n-3}DPA (**5**) with synthetic material. Results are representative of *n* = 4.

findings demonstrate that **5** displayed both potent anti-inflammatory and pro-resolving actions, confirming the potent immunoresolvent properties of PD1_{n-3}DPA (**5**).

The complete structure elucidation and a stereocontrolled total synthesis of PD1_{n-3}DPA have been reported. These efforts unambiguously confirmed the structure of PD1_{n-3}DPA (**5**) to be (7*Z*,10*R*,11*E*,13*E*,15*Z*,17*S*,19*Z*)-10,17-dihydrodocosa-7,11,13,15,19-pentaenoic acid. The synthetic material was matched to that of endogenously produced PD1_{n-3}DPA (**5**). Compound **5** displayed potent anti-inflammatory and pro-resolving activities characteristic for members of the SPM class of natural products. The dihydroxy-polyunsaturated fatty acid **5** is a congener with the DHA-derived protectin D1 (**3**).^{7–11,20} Protectin D1 (**3**) exhibits potent protective activity in vivo in models of inflammatory diseases^{5,20} as well as other interesting biological actions in organ protection³⁰ and reduces pain.³⁵ Further biological evaluations of PD1_{n-3}DPA (**5**) are ongoing and will be reported separately.

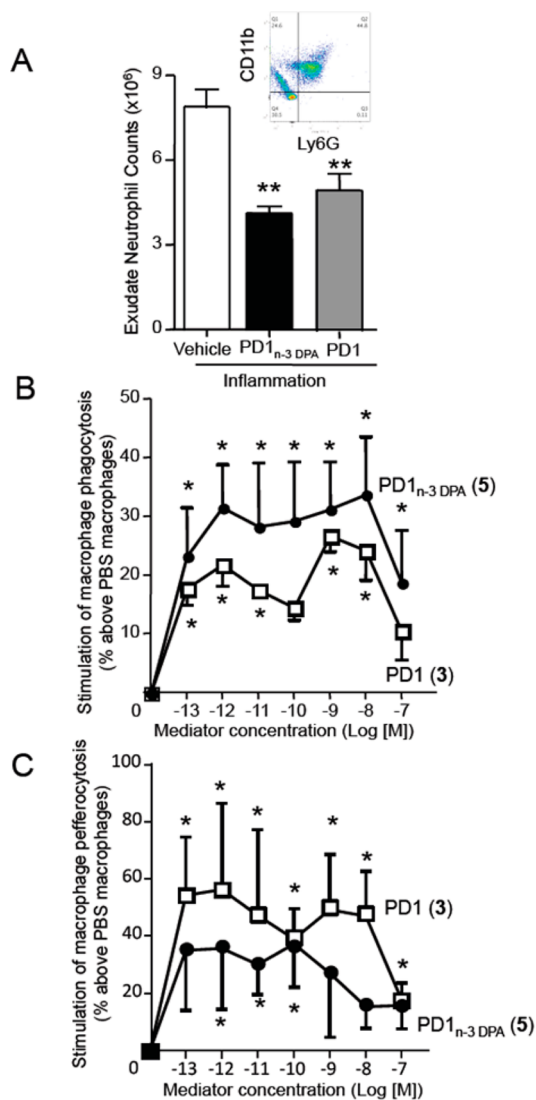


Figure 3. PD1_{n-3}DPA (5) displays potent anti-inflammatory and pro-resolving actions. PD1_{n-3}DPA, (5), PD1 (3), or vehicle (saline containing 0.01% EtOH) were administered iv 5 min prior to ip administration of zymosan (1 mg). Exudates were collected at 4 h, and the number of infiltrated neutrophils was determined by flow cytometry (top right inset) and light microscopy. Results are mean \pm SEM. $n = 4$ mice per treatment (** $p < 0.01$ vs vehicle group). (B, C) Macrophages were incubated with vehicle (0.1% EtOH in PBS), PD1_{n-3}DPA (5) (100 nM to 10 pM), or PD1 (3) (100 nM to 10 pM; 15 min, 37 °C, pH = 7.45) prior to addition of (B) fluorescently labeled zymosan (1:10 macrophages to zymosan) or (C) fluorescently labeled apoptotic human neutrophils. After 60 min (37 °C, pH = 7.45), the incubation was stopped, extracellular fluorescence quenched using trypan blue, and phagocytosis assessed using a SpectraMax M3 plate reader. Results are mean \pm SEM. $n = 4$ macrophage preparations (* $p < 0.05$ vs PBS-incubated macrophages).

EXPERIMENTAL SECTION

General Experimental Procedures. Unless stated otherwise, all commercially available reagents and solvents were used in the form they were supplied without any further purification. The stated yields are based on isolated material. Zymosan was purchased from Sigma-Aldrich. Optical rotations were measured using a 1 mL cell with a 1.0 dm path length on a Perkin-Elmer 341 polarimeter. The UV/vis spectra from 190 to 900 nm were recorded using a Biochrom Libra S32PC spectrometer using quartz cuvettes. IR spectra (4000–600 cm^{-1}) were obtained on a Perkin-Elmer Spectrum BX series FT-IR

spectrophotometer. NMR spectra were recorded on a Bruker DRX500 or a Bruker AVII400 spectrometer at 500 or 400 MHz, respectively, for ^1H NMR and at 126 or 101 MHz, respectively, for ^{13}C NMR. Spectra are referenced relative to the central residual protium solvent resonance in ^1H NMR (CDCl_3 $\delta = 7.27$ and $\text{MeOH-}d_4$ $\delta = 3.31$) and the central carbon solvent resonance in ^{13}C NMR (CDCl_3 $\delta = 77.00$ ppm and $\text{MeOH-}d_4$ $\delta = 49.00$). Mass spectra were recorded at 70 eV on a Waters Prospec Q spectrometer using EI, ES, or CI as the method of ionization. High-resolution mass spectra were recorded on a Waters Prospec Q spectrometer using EI or ES as the methods of ionization. Thin-layer chromatography was performed on silica gel 60 F254 aluminum-backed plates fabricated by Merck. Flash column chromatography was performed on silica gel 60 (40–63 μm) produced by Merck. HPLC analyses were performed on an Agilent Technologies 1200 Series instrument with diode array detector set at 254 nm and equipped with a C18 stationary phase (Eclipse XDB-C18 5 μm , 4.6 \times 150 mm), applying the conditions stated. Diastereomeric ratios reported in this paper have not been validated by calibration.³⁶

Methyl (10R,7Z,11E,13E)-14-Bromo-10-((tert-butylidimethylsilyloxy)tetradeca-7,11,13-trienoate (14). To the Wittig salt 7 (1.67 g, 3.13 mmol, 1.0 equiv) in THF (47 mL) was added NaHMDS (0.6 M in toluene, 1.0 equiv) at -78 °C, and the mixture was stirred for 60 min at that temperature. Aldehyde 12 was added. The solution was allowed to warm slowly to room temperature in a dry ice/acetone bath and stirred for 24 h before it was quenched with phosphate buffer (45 mL, pH = 7.2). Et_2O (60 mL) was added, and the phases were separated. The H_2O phase was extracted with Et_2O (2×60 mL) and the combined organic layers were dried (Na_2SO_4), before the solvent was evaporated. The crude product was purified by column chromatography on silica (hexanes/ EtOAc , 97:3) to afford the title compound 14 as a colorless oil. Yield: 754 mg (54% over two steps). $[\alpha]_D^{20} -18$ (c 0.09, MeOH); ^1H NMR (400 MHz, CDCl_3) δ 6.68 (dd, $J = 13.4, 10.9$ Hz, 1H), 6.27 (d, $J = 13.5$ Hz, 1H), 6.09 (dd, $J = 15.2, 10.9$ Hz, 1H), 5.71 (dd, $J = 15.3, 9.6$ Hz, 1H), 5.48–5.29 (m, 1H), 4.13 (dd, $J = 6.0, 1.4$ Hz, 1H), 3.66 (s, 3H), 2.30 (t, $J = 7.5$ Hz, 2H), 2.28–2.17 (m, 2H), 2.05–1.95 (m, 2H), 1.62 (p, $J = 7.4$ Hz, 2H), 1.40–1.25 (m, 4H), 0.89 (s, 9H), 0.04 (s, 3H), 0.02 (s, 3H); ^{13}C NMR (101 MHz, CDCl_3) δ 174.3, 138.1, 137.2, 131.9, 126.6, 125.2, 108.2, 72.7, 51.6, 36.3, 34.2, 29.4, 29.0, 27.4, 26.0 (3C), 25.0, 18.4, -4.4 , -4.6 ; HREIMS m/z 447.1751 [$\text{M} + \text{H}$]⁺ (calcd for $\text{C}_{21}\text{H}_{38}^{81}\text{BrO}_3\text{Si}$, 447.1753); TLC (hexanes/ EtOAc , 95:5, CAM stain) $R_f = 0.21$; diastereomeric ratio (>98%) was determined by HPLC analysis (Eclipse XDB-C18, MeOH/ H_2O , 92:8, 1.1 mL/min); t_r (minor) = 8.41 and 8.74 min and t_r (major) = 9.30 min.

Methyl (7Z,10R,11E,13E,17S,19Z)-10,17-Bis((tert-butylidimethylsilyloxy)docosa-7,11,13,19-tetraen-15-ynoate (18). To a solution of vinyl bromide 14 (285 mg, 0.64 mmol, 1.0 equiv) in Et_2NH (1.5 mL) and benzene (0.6 mL) was added Pd(PPh_3)₄ (22 mg, 0.019 mmol, 3 mol %), and the reaction was stirred for 45 min in the dark. CuI (6 mg, 0.032 mmol, 5 mol %) in a minimum amount of Et_2NH was added, followed by dropwise addition of alkyne 15 (153 mg, 0.64 mmol, 1.0 equiv) in Et_3N (1.5 mL). After stirring at ambient temperature for 20 h, the reaction was quenched by saturated NH_4Cl (15 mL). Et_2O (15 mL) was added, and the phases were separated. The H_2O phase was extracted with Et_2O (2×15 mL) and the combined organic layers were dried (Na_2SO_4), before the solvent was evaporated. The crude product was purified by column chromatography on silica (hexanes/ EtOAc , 9:1) to afford the title compound 18 as a pale yellow oil. Yield: 387 mg (92%); $[\alpha]_D^{20} -21$ (c 0.08, MeOH); ^1H NMR (400 MHz, CDCl_3) δ 6.51 (dd, $J = 15.5, 10.9$ Hz, 1H), 6.18 (ddd, $J = 14.2, 10.3, 1.0$ Hz, 1H), 5.75 (dd, $J = 15.2, 5.9$ Hz, 1H), 5.62–5.31 (m, 5H), 4.47 (td, $J = 6.6, 1.8$ Hz, 1H), 4.21–4.12 (m, 1H), 3.66 (t, 3H), 2.44 (t, $J = 7.9$ Hz, 2H), 2.30 (t, $J = 7.6$ Hz, 2H), 2.28–2.15 (m, 2H), 2.11–1.94 (m, 4H), 1.63 (q, $J = 7.4$ Hz, 2H), 1.40–1.25 (m, 4H), 0.96 (t, $J = 7.5$ Hz, 3H), 0.91 (s, 9H), 0.89 (s, 9H), 0.12 (d, $J = 8.3$ Hz, 6H), 0.03 (d, $J = 8.3$ Hz, 6H); ^{13}C NMR (101 MHz, CDCl_3) δ 174.3, 141.2, 139.3, 134.4, 131.9, 128.6, 125.4, 124.1, 110.7, 93.4, 83.6, 72.9, 63.7, 51.6, 36.8, 36.4, 34.2, 29.4, 29.0, 27.4, 26.0 (3C), 26.0 (3C), 25.0, 20.9, 18.5, 18.4, 14.4, -4.3 (2C), -4.6 , -4.8 ; HRESTOFMS m/z 625.4084 [$\text{M} + \text{Na}$]⁺ (calcd for

C₃₅H₆₂O₄Si₂Na, 625.4085); TLC (hexanes/EtOAc, 9:1, CAM stain) R_f = 0.63.

Methyl (7Z,10R,11E,13E,17S,19Z)-10,17-Dihydroxydocosa-7,11,13,19-tetraen-15-ynoate (19). TBAF (704 mg, 2.70 mmol, 5.0 equiv, 1.0 M in THF) was added to a solution of TBS-protected alcohol **18** (325 mg, 0.54 mmol, 1.0 equiv) in THF (6.9 mL) at 0 °C. The reaction was stirred for 2.5 h before it was quenched with phosphate buffer (pH = 7.2, 2.8 mL). Brine (30 mL) and EtOAc (30 mL) were added, and the phases were separated. The H₂O phase was extracted with EtOAc (2 × 30 mL) and the combined organic layers were dried (Na₂SO₄) before the solvent was evaporated. The crude product was purified by column chromatography on silica (hexanes/EtOAc, 7:3) to afford the title compound **19** as a pale yellow oil. Yield: 202 mg (85%); $[\alpha]_D^{20}$ -16 (c 0.06, MeOH); ¹H NMR (400 MHz, CDCl₃) δ 6.55 (dd, *J* = 15.6, 11.0 Hz, 1H), 6.27 (dd, *J* = 14.9, 11.6 Hz, 1H), 5.81 (dd, *J* = 15.2, 6.1 Hz, 1H), 5.67–5.49 (m, 3H), 5.48–5.31 (m, 2H), 4.52 (td, *J* = 6.4, 1.9 Hz, 1H), 4.25–4.16 (m, 1H), 3.68 (t, 3H), 2.49 (t, *J* = 6.9 Hz, 2H), 2.35–2.26 (m, 4H), 2.14–1.99 (m, 4H), 1.98 (d, *J* = 5.4 Hz, 1H), 1.71 (bs, 1H); 1.62 (p, *J* = 7.5 Hz, 2H), 1.43–1.27 (m, 4H), 0.97 (t, *J* = 7.5 Hz, 3H); ¹³C NMR (101 MHz, CDCl₃) δ 174.4, 141.5, 138.4, 136.1, 133.8, 129.4, 124.3, 122.9, 110.9, 92.6, 84.1, 71.7, 62.7, 51.7, 35.8, 35.5, 34.2, 29.3, 28.9, 27.4, 25.0, 21.0, 14.4; HRESTOFMS *m/z* 397.2354 [M + Na]⁺ (calcd for C₂₃H₃₄O₄Na, 397.2355); TLC (hexanes/EtOAc, 7:3, CAM stain) R_f = 0.26.

Methyl (7Z,10R,11E,13E,15Z,17S,19Z)-10,17-Dihydroxydocosa-7,11,13,15,19-pentaenoate (6). To a solution of alkyne **19** (30 mg, 0.08 mmol) in EtOAc/pyridine/1-octene (1.65 mL, 10:1:1) under argon was added Lindlar's catalyst (45 mg), and the flask was evacuated and filled with argon. The reaction was stirred for 60 h at ambient temperature under a balloon of hydrogen gas until completion. The reaction mixture was loaded directly onto a silica gel column and purified by chromatography (hexanes/EtOAc, 9:1) to afford the title compound **6** as a pale yellow oil. Yield: 15 mg (50%). $[\alpha]_D^{20}$ = -19 (c 0.08, MeOH); UV (MeOH) λ_{max} 262 (log ϵ 4.52), 271 (log ϵ 4.60) and 282 nm (log ϵ 4.53); ¹H NMR (500 MHz, MeOH-*d*₄) and ¹³C NMR (126 MHz, MeOH-*d*₄), see Table 1; HRESTOFMS *m/z* 399.2511 [M + Na]⁺ (calcd for C₂₃H₃₆O₄Na, 399.2506); TLC (hexanes/EtOAc, 6:4, CAM stain) R_f = 0.16; diastereomeric ratio was determined by HPLC (Eclipse XDB-C18, MeOH/H₂O, 8:2, 0.8 mL/min); t_r (minor) = 6.67 min and t_r (major) = 8.31 min.

Protectin D1_{n-3} DPA (5). Methyl ester **6** (14 mg, 0.04 mmol) was dissolved in MeOH/H₂O, 1:1 (35 mL), and cooled to 0 °C. LiOH (1.0 M, 2.2 mL) was added dropwise. The reaction mixture was stirred at the above-mentioned temperature for 48 h, after which a saturated solution of NaH₂PO₄ (3.3 mL) was added. Next, NaCl (10.0 g) was added followed by EtOAc (50 mL). The organic phase was decanted, dried (Na₂SO₄), and concentrated in vacuo, affording the title compound **5** as a colorless oil. Yield: 9.5 mg (71%); $[\alpha]_D^{20}$ -28 (c 0.1, MeOH); UV (MeOH) λ_{max} 262 (log ϵ 4.53), 271 (log ϵ 4.60), 282 nm (log ϵ 4.54); ¹H NMR (400 MHz, MeOH-*d*₄) δ 6.52 (dd, *J* = 13.7, 11.1 Hz, 1H), 6.31–6.20 (m, 2H), 6.08 (t, *J* = 11.0 Hz, 1H), 5.74 (dd, *J* = 14.5, 6.6 Hz, 1H), 5.50–5.30 (m, 5H), 4.56 (dt, *J* = 8.6, 6.3 Hz, 1H), 4.15–4.09 (m, 1H), 2.40–2.17 (m, 6H), 2.10–2.02 (m, 4H), 1.60 (p, *J* = 7.4 Hz, 2H), 1.42–1.30 (m, 4H), 0.97 (t, *J* = 7.5 Hz, 3H); ¹³C NMR (101 MHz, MeOH-*d*₄) δ 178.2, 138.0, 134.9, 134.8, 134.7, 132.9, 131.3, 130.6, 128.9, 126.2, 125.3, 73.1, 68.6, 36.4, 36.4, 35.4, 30.4, 29.9, 28.3, 26.2, 21.7, 14.6; HRESTOFMS *m/z* 361.2378 [M - H]⁺ (calcd for C₂₂H₃₃O₄, 361.2385); TLC (Et₂O with a drop of AcOH, CAM stain) R_f = 0.34. The purity (>98%) was determined by HPLC analysis (Eclipse XDB-C18, MeOH/3.3 mM HCOOH in H₂O, 7:3, 1.0 mL/min); t_r (major) = 9.52 min and t_r (minor) = 13.49 min.

Biogenic Synthesis and Identification of 16,17-Epoxy PD_{n-3} DPA. In brief, soybean lipoxygenase (50 U/100 mL) was incubated with *n*-3 DPA (0.2 μM in 200 μL of borate buffer (pH = 8.2)) at 4 °C. After 20 s, eight volumes of acidified MeOH (apparent pH ~3.5) were added. The resulting products were investigated by target lipid mediator metabololipidomics as previously described.³²

Lipid Mediator Metabololipidomics. Matching of synthetic **5** with endogenous products was conducted as previously reported.³¹

Briefly, as developed for other lipid mediators, all samples for LC-MS/MS-based lipidomics were subject to solid-phase extraction. Prior to sample extraction, *d*₄-LTB₄ (500 pg) was added. Extracted samples were analyzed with an LC-MS system, a QTrap 5500 (ABSciex) equipped with a Shimadzu SIL-20AC HT autosampler and LC-20AD LC pumps. An Agilent Eclipse Plus C18 column (100 mm × 4.6 mm × 1.8 μm) was used with a gradient of MeOH/H₂O/acetic acid of 55:45:0.01 (v/v/v) to 100:0:0.01 at a 0.4 mL/min flow rate. To monitor targeted SPMs, we used multiple reaction monitoring (MRM) with signature ion fragments for each molecule (at least six diagnostic ions).³² GC-MS analyses were carried out as previously reported.⁹

Preparation of Naturally Occurring PD1_{n-3} DPA (5). Male FVB mice (6 to 8 weeks old) purchased from Charles River Laboratories were fed ad libitum laboratory rodent diet 20-5058 (Lab Diet, Purina Mills). All animal experimental procedures were approved by the Standing Committee on Animals of Harvard Medical School (protocol no. 02570) and complied with institutional and U.S. National Institutes of Health (NIH) guidelines. Peritonitis was induced by zymosan injection (1 mg/mL) intraperitoneally (ip), and exudates were obtained 4 h later. Human macrophages were prepared from peripheral blood mononuclear cells following literature protocols.³¹ Macrophages were suspended in DPBS^{+/+} (5 × 10⁷ cells/mL) and incubated with *n*-3 DPA (1 μM) and 0.1 mg of serum-treated zymosan (37 °C, 30 min, pH = 7.45). The incubations were stopped with 2 mL of ice-cold MeOH, and mediators extracted over C18 columns as described above.

Anti-inflammatory and Pro-resolving Actions. Mice were administered intravenously (iv) vehicle (saline containing 0.1% EtOH), PD1_{n-3} DPA (**5**) (10 ng/mouse), or protectin D1 (**3**) for the purpose of direct comparison (10 ng/mouse) 5 min prior to ip zymosan administration (1 mg). After 4 h the exudates were collected and the number of extravasated neutrophils was determined using Turks solution and flow cytometry.¹⁹ Human macrophages and peripheral blood neutrophils were prepared; then phagocytosis and efferocytosis were assessed as described in ref 19. Briefly, cells were incubated with vehicle (0.1% EtOH in DPBS), PD1_{n-3} DPA (**5**), or protectin D1 (**3**) at the indicated concentrations for 15 min at 37 °C; then FITC-labeled zymosan- or bisbenzimidazole-labeled apoptotic neutrophils were added and cells incubated for 60 min at 37 °C. Phagocytosis was assessed using an M3 SpectraMax plate reader.

■ ASSOCIATED CONTENT

■ Supporting Information

Experimental procedures and characterization data of synthetic intermediates **7–19**, PD1_{n-3} DPA (**5**), and its methyl ester **6**, ¹H, ¹³C, and 2D-NMR spectra data, HRMS and UV/vis spectra, HPLC analyses of synthetic compounds as well as LC/MS/MS data and GC/MS chromatograms of endogenous PD1_{n-3} DPA (**5**) and its bis-TMS ether of methyl ester **6** are available free of charge via the Internet at <http://pubs.acs.org>.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare the following competing financial interest(s): J.D. and C.N.S. have filed patents on PD1_{n-3} DPA (**5**) and related compounds. C.N.S.'s interests are reviewed and

are managed by BWH and Partners HealthCare in accordance with their conflict of interest policies.

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