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ABSTRACT: The polyunsaturated lipid mediator PD1n-3 DPA (5) was recently isolated from self-resolving inflammatory exudates of 5 and human macrophages. Herein, the first total synthesis of PD1n-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 (7Z,10R,11E,13E,15Z,17S,19Z)-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 PD1n-3 DPA (5) demonstrated potent anti-inflammatory properties together with pro-resolving actions stimulating human macrophage phagocytosis and effectorcytosis. 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 system9,10 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 \((7Z,10Z,13Z,16Z,19Z)\)-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 \((7Z,11E,13E,15Z,17S,19Z)-10,17\)-dihydroxydocosahexaenoic acid and denoted PD1\(_{n-3}\) DPA (5), given its relation to PD1 (3)\(^{20}\) (Figure 1).

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 \((7S,8E,10Z,13Z,15E,17S,19Z)\)-7,17-dihydroxydocosahexaenoic 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 oxidation\(^{23}\) of 8 yielded lactone 9, which was reacted with MeOH and catalytic amounts of H\(_2\)SO\(_4\) 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 \((3R,4E,6E)-7\)-bromo-3-\(((\text{tert-butyl}dime)syl)\)oxyhepta-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](image-url)
after reaction with NaHMDS in THF at \(-78^\circ C\), 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 \(^1\)H 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 reaction\(^{26}\) at ambient temperature in the presence of catalytic Pd(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 \(^\circ\)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 reaction\(^{28}\) with high stereoselectivity, as only one diastereomer was detected by HPLC and \(^1\)H NMR analyses. Finally, hydrolysis of the methyl ester 6 at 0 \(^\circ\)C with dilute aqueous LiOH in MeOH followed by mild acidic workup with aqueous Na\(_2\)HPO\(_4\) resulted in a 71\% yield of PD1\(_{n-3}\)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 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, \(J = 14.4\) Hz) and 6.07 ppm (t, 1H, \(J = 11.0\) 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,E,Z-triene moiety. Moreover, two signals from the hydrogen atoms attached to the carbonyl carbon atoms were observed as expected with signals at 4.12 (m, 1H) and 4.56 ppm (dt, 1H, \(J = 8.9, 6.8\) 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 \(^1\)H and \(^1\)C NMR spectra, respectively. The COSY spectrum was used for assigning vicinal hydrogen-nitrogen coupling patterns of the formed products, four structures were assigned (Figure S-25). These results render support for the proposed biosynthetic pathway\(^{19}\) as depicted in Figure 2, involving an acid-catalyzed ring-opening of the 16,17-epoxyPD1\(_{n-3}\)DPA. In addition, these observations are in accordance with the reaction pathway reported by Corey and Mehrotra\(^{33}\) as well as enzyme-catalyzed mechanisms in the biosynthesis of other pro-resolving mediators.\(^{14}\)

In order to determine whether synthetic 5 matched endogenous PD1\(_{n-3}\)DPA (5), authentic 5 from murine self-resolving exudates as well as human macrophages was employed.\(^{19}\) Figure 2A shows human macrophage PD1\(_{n-3}\)DPA (5) from n-3 DPA with a retention time \((R)\) of 12.4 min, together with its natural isomers. Figure 2B depicts endogenous PD1\(_{n-3}\)DPA (5) from mouse peritoneal exudates that also displayed \(R\) = 12.4 min. This is shown together with its natural isomers produced in mouse peritoneal exudates. The chromatographic behavior of synthetic 5 (\(R\) = 12.4 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...
amounts. Altogether, Figure 2 demonstrates that synthetic S coelutes with endogenously obtained S. In addition, the MS/MS spectra for both natural and synthetic S were essentially identical (Figure S-24).

Having established that the physical properties of synthetic S matched those of endogenous S, we next required the confirmation of its anti-inflammatory and pro-resolving actions. Administration of synthetic S 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). The human macrophage phagocytosis and efferocytosis (clearance of apoptotic neutrophils) are key processes in the resolution of inflammation and defining actions of SPMs. The ability of synthetic S in this system to stimulate human macrophage phagocytosis and efferocytosis was investigated. Incubation of synthetic S with human macrophages and increasing concentrations of S led to an increase in macrophage phagocytosis of both fluorescence-labeled yeast cell particles (zymosan) and apoptotic neutrophils. These actions were shared with the DHA-derived protectin D1 (3) (Figure 3B). In addition, 5 displayed potent efferocytosis effects against apoptotic human neutrophils (Figure 3C). 5 displayed potent 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 findings demonstrate that 5 displayed both potent anti-inflammatory and pro-resolving actions, confirming the potent immunoresolvent properties of 5.

The complete structure elucidation and a stereocontrolled total synthesis of 5 have been reported. These efforts unambiguously confirmed the structure of 5 to be (7Z,10R,11E,13E,15Z,17S,19Z)-10,17-di-hydroxydocosa-7,11,13,15,19-pentaenoic acid. The synthetic material was matched to that of endogenously produced 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 is a congener with the DHA-derived protectin D1 (3) which exhibits potent protective activity in vivo in models of inflammatory diseases, as well as other interesting biological actions in organ protection and reduces pain. Further biological evaluations of 5 are ongoing and will be reported separately.

Figure 2. Endogenous PD1n-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) PD1n-3 DPA (5) from human macrophages (5 × 107 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 PD1n-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, λmax MeOH ± 1 nm). (D) Co-injection of resolving exudate endogenous PD1n-3 DPA (5) with synthetic material. Results are representative of n = 4.

Table 1. Compilation of 1H and 13C NMR Data of the Methyl Ester 6

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MeOH-d4 was used as solvent. See Figure 1 for atom labeling.

1H and 13C NMR spectroscopy was performed in MeOD-d4 at 298 K.

δH were assigned using the center of the COSY and HSQC peak intensities.

The complete structure elucidation and a stereocontrolled total synthesis of 5 have been reported. These efforts unambiguously confirmed the structure of 5 to be (7Z,10R,11E,13E,15Z,17S,19Z)-10,17-di-hydroxydocosa-7,11,13,15,19-pentaenoic acid. The synthetic material was matched to that of endogenously produced 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 is a congener with the DHA-derived protectin D1 (3) which exhibits potent protective activity in vivo in models of inflammatory diseases, as well as other interesting biological actions in organ protection and reduces pain. Further biological evaluations of 5 are ongoing and will be reported separately.
Figure 3. PD1b,3_DPA (5) displays potent anti-inflammatory and pro-resolving actions. PD1b,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 ± SEM. n = 4 mice per treatment (*p < 0.01 vs vehicle group). (B, C) Macrophages were incubated with vehicle (0.1% EtOH in PBS), PD1b,3_DPA (5) (100 nM to 10 μM), or PD1 (3) (100 nM to 10 μM; 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 ± 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 Spectra BX series FT-IR spectrophotometer. NMR spectra were recorded on a Bruker DRX500 or a Bruker AVIII400 spectrometer at 500 or 400 MHz, respectively, for 1H NMR and at 126 or 101 MHz, respectively, for 13C NMR. Spectra are referenced relative to the central residual proton solvent resonance in 1H NMR (CDCl₃, δ = 7.27 and MeOH-d₄, δ = 3.31) and the central carbon solvent resonance in 13C NMR (CDCl₃, δ = 77.00 ppm and MeOH-d₄ = δ = 49.00). Mass spectra were recorded at 7.0 V 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 method 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 × 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-10,17-Bis((tert-butyl(dimethyl)silyl)oxy)tetradeca-7,11,13,19-tetraen-15-ynoate (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). Et₂O (60 mL) was added, and the phases were separated. The H₂O phase was extracted with Et₂O (2 × 60 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, 97:3) to afford the title compound 14 as a colorless oil. Yield: 754 mg (54% over two steps). [α]D = −18 (c 0.09, MeOH); 1H NMR (400 MHz, CDCl₃) 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 = 4.6 Hz, 2H), 1.40–1.25 (m, 4H), 0.89 (s, 9H), 0.04 (s, 3H), 0.02 (s, 3H); 13C NMR (101 MHz, CDCl₃) 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 447.1753 [M + H]⁺ (calcd for C₄₁H₆₁BrO₃Si, 447.1753); TLC (hexanes/EtOAc, 95:5; CAM stain) Rf = 0.21; diastereomeric ratio (>98%) was determined by HPLC analysis (Eclipse XDB-C18, MeOH/H₂O, 92.8:1.1 mL/min; tₚ(minor) = 8.41 and 8.74 min and tₚ(major) = 9.30 min. Methyl (7Z,10R,11E,13E,17S,19Z)-10,17-Bis((tert-butyl(dimethyl)silyl)oxy)ocosa-7,11,13,19-tetraen-15-ynoate (18). To a solution of vinyl bromide 14 (285 mg, 0.64 mmol, 1.0 equiv) in Et₂NH (1.5 mL) and benzene (0.6 mL) was added Pd(PPh₃)₄ (22 mg, 0.019 mmol, 3 mol %), and the reaction was stirred for 45 min in the dark. Cul (6 mg, 0.032 mmol, 5 mol %) in a minimum amount of Et₂NH was added, followed by dropwise addition of allyne 15 (153 mg, 0.66 mmol, 1.0 equiv in Et₂N (1.5 mL). After stirring at ambient temperature for 20 h, the reaction was quenched by saturated NH₄Cl (15 mL). Et₂O (15 mL) was added, and the phases were separated. The H₂O phase was extracted with Et₂O (3 × 15 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, 9:1) to afford the title compound 18 as a pale yellow oil. Yield: 387 mg (92%); [α]D = −21 (c 0.08, MeOH); 1H NMR (400 MHz, CDCl₃) 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 (ddd, J = 15.2, 5.9 Hz, 1H), 5.62–5.31 (m, 2H), 4.47 (td, J = 6.6, 1.8 Hz, 1H), 4.21–4.12 (m, 1H), 3.66 (t, J = 4.6 Hz, 2H), 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, 5H), 0.96 (t, J = 7.5 Hz, 3H), 0.91 (s, 3H), 0.89 (s, 9H), 0.12 (d, J = 8.3 Hz, 6H), 0.03 (d, J = 8.3 Hz, 1H); 13C NMR (101 MHz, CDCl₃) 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, 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 [M + Na]⁺ (calcd for 914 dx.doi.org/10.1021/np4009865 J. Nat. Prod. 2014, 77, 910–916
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<sub>4</sub>-LTB<sub>4</sub> (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:H<sub>2</sub>O/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).<sup>32</sup> GC-MS analyses were carried out as previously reported.<sup>3</sup>

Preparation of Naturally Occurring PD<sub>1n</sub>-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) in intraperitoneally (ip) and exudates were obtained 4 h later. Human macrophages were prepared from peripheral blood mononuclear cells following literature protocols.<sup>31</sup> Macrophages were cultured in DBPS<sup>−</sup> (5 × 10<sup>4</sup> 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), PD<sub>1n</sub>-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 a Turks solution and flow cytometry. 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<sup>−</sup>), PD<sub>1n</sub>-3 DPA (5), or protectin D1 (5) at the indicated concentrations for 15 min at 37 °C; then FITC-labeled zymosan- or bisbenzimide-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, PD<sub>1n</sub>-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 PD<sub>1n</sub>-3 DPA (5) and its bis-2TMS ether of methyl ester 6 are available free of charge via the Internet at http://pubs.acs.org.

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**Notes**
The authors declare the following competing financial interests(s): J.D. and C.N.S. have filed patents on PD<sub>1n</sub>-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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