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Citation

Published Version
doi:10.1021/np4009865

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Accessibility
Total Synthesis of the Lipid Mediator PD1$_{n-3}$ DPA: Configurational Assignments and Anti-inflammatory and Pro-resolving Actions

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§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 (7Z,10R,11E,13E,15Z,19Z)-10,17-dihydroxydocos-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 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 PUFAs 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 systems9,10 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
subject of many pharmacological studies for the development of potential new anti-inflammatory drugs\textsuperscript{14,15} and some have already been the subject of clinical trials.\textsuperscript{15} These autacoids exhibit stereochemically selective modes of actions, reflecting their routes of biosynthesis.\textsuperscript{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\textit{Z},10\textit{Z},13\textit{Z},15\textit{Z},19\textit{Z})-7,17-dihydroxydocosao-8,10,13,15,19-pentaeanoic acid, produced under the same experimental conditions.\textsuperscript{15} 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.\textsuperscript{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.

\section*{RESULTS AND DISCUSSION}

The synthesis of PD1\textsubscript{n-3 DPA} (5) commenced with the preparation of the Wittig salt 7 in four steps from cycloheptanone (8). First, a Baeyer–Villiger oxidation\textsuperscript{23} of 8 yielded lactone 9, which was reacted with MeOH and catalytic amounts of H\textsubscript{2}SO\textsubscript{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 (3\textit{R},4\textit{E},6\textit{E})-7-bromo-3-((tert-butyldimethylsilyl)oxy)hepta-4,6-dienal (12) was prepared as previously reported from salt 13.\textsuperscript{24} Aldehyde 12 was reacted with the ylide of 7, the latter obtained

Figure 1. Proposed biosynthesis of PD1\textsubscript{n-3 DPA} (5).

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

The initial structural assignment of the n-3 DPA-derived compound PD1\textsubscript{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\textsubscript{n-3 DPA} or (7\textit{S},8\textit{E},10\textit{Z},13\textit{Z},15\textit{E},17\textit{S},19\textit{Z})-7,17-dihydroxydocosa-8,10,13,15,19-pentaeanoic acid, produced under the same experimental conditions.\textsuperscript{15} 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.\textsuperscript{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.

Scheme 1. Synthesis of Wittig Salt 7 and Intermediate 14
after reaction with NaHMDS in THF at ~78 °C, in a Z-selective Wittig reaction. After purification using silica gel chromatography, pure vinylc 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 glycol (17). Then vinylc bromide ester 14 and alkyne 15 were reacted in a Sonogashira reaction at ambient temperature in the presence of catalytic Pd(PPh3)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 °C gave an 85% yield of the diol. 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 with high stereoselectivity, as well as one diastereomer was detected by HPLC and 1H NMR analyses. Finally, hydrolysis of the methyl ester 6 at 0 °C with dilute aqueous LiOH in MeOH followed by mild acidic washup with aqueous NaH2PO4 resulted in a 71% yield of PD1n-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-d4 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,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 Z-double bonds, respectively. The data from the 1H and 13C 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 PD1n-3 DPA (5) were compared. For synthetic 5, λmax MeOH absorbances were observed at 262, 271, and 282 nm; all in excellent agreement with the endogenous product (261, 271, 282 nm, λmax MeOH), as well as with literature values. 5 Synthetical 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 – CH2, 489 = M – OCH3, 430 = M – OTMS, 340 = M – 2xOTMS, and 73 = TMS.

Experiments supporting the proposed biosynthetic pathway in Figure 2 were then performed. Soybean lipoxigenase was incubated with n-3 DPA in borate buffer (pH = 8.0) at ambient temperature essentially as described. 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. From the MS/MS fragmentation patterns of the formed products, four structures were assigned (Figure S-26). These results render support for the proposed biosynthetic pathway, as depicted in Figure 2, involving an acid-catalyzed ring-opening of the 16,17-epoxyPD1n-3 DPA. In addition, these observations are in accordance with the reaction pathway reported by Corey and Mehrotra as well as enzyme-catalyzed mechanisms in the biosynthesis of other pro-resolving mediators.

In order to determine whether synthetic 5 matched endogenous PD1n-3 DPA (5), authentic 5 from murine self-resolving exudates as well as human macrophages was employed. Figure 2A shows human macrophage PD1n-3 DPA (5) from n-3 DPA with a retention time (tR) of 12.4 min, together with its natural isomers. Figure 2B depicts endogenous PD1n-3 DPA (5) from mouse peritoneal exudates that also displayed tR = 12.4 min. This is shown together with its natural isomers produced in mouse peritoneal exudates. The chromatographic behavior of synthetic 5 (tR = 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 to human macrophages to confirm its identity.
Table 1. Compilation of $^1$H and $^{13}$C NMR Data of the Methyl Ester 6

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$^b$ MeOH-d$_4$ was used as solvent. See Figure 1 for atom labeling.
$^c$ Measured at 125 MHz.
$^d$ Measured at 500 MHz. HMBC correlations are from proton(s) stated to the indicated carbon(s). The ppm values listed above for $\delta_{H}$ were assigned using the center of the COSY and HSQC peak intensities.

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$^5$ 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, $\lambda_{max}$ MeOH ± 1 nm). (D) Co-injection of resolving exude 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 stereocoupled total synthesis of PD1$_{n,3}$DPA have been reported. These efforts unambiguously confirmed the structure of PD1$_{n,3}$DPA (5) to be (7Z,10R,11E,13E,15Z,17S,19Z)-10,17-dihydroxydocos-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) and will be reported separately.
Macrophages were incubated with vehicle (0.1% EtOH in PBS), SEM. ± cytometry (top right inset) and light microscopy. Results are mean using trypan blue, and phagocytosis assessed using a SpectraMax M3 the number of in containing 0.01% EtOH) were administered iv 5 min prior to ip ° labeled zymosan (1:10 macrophages to zymosan) or (C) ° pH = 7.45), the incubation was stopped, extracellular ° 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-((tert-butyldimethylsilyloxy)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), 5.48–5.59 (m, 1H), 4.13 (dd, J = 6.0, 1.4 Hz, 1H), 3.66 (s, 3H), 3.20 (t, J = 7.5 Hz, 2H), 2.28–2.17 (m, 2H), 2.05–1.95 (m, 2H), 1.62 (p, J = 7.5 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, 51.6, 36.3, 34.2, 29.4, 29.0, 27.4, 26.0 (3C), 25.0, 22.8, 21.1, 19.5, 18.5, 18.4, 14.4, 14.4, 14.3 (2C), −4.6, −4.8; HREIMS m/z 447.1753 [M + H]+ (calcd for C₂₅H₄₇BrO₃Si, 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); t₁(minor) = 8.41 and 8.74 min and t₁(major) = 9.30 min.

**Methyl (7Z,10R,11E,13E,17S,19Z)-10,17-Bis((tert-butyldimethylsilyloxy)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 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. The soln (6 mg, 0.032 mmol, 5 mol %) in a minimum amount of Et₂NH was added, followed by dropwise addition of allyne 15 (153 mg, 0.64 mmol, 1.0 equiv in Et₂N (1.5 mL). After stirring at ambient temperature for 2 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 6.61 (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, SH), 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, 3H); 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, 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; HREIMS m/z 625.4084 [M + Na]+ (calcd for

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**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 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 protium 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 7.0V on a Waters Prospe Q spectrometer using EI, ES, or CI as the method of ionization. High-resolution mass spectra were recorded on a Waters Prospe 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.
Methyl 7Z,10R,11E,13E,17,19-dihydroxydoco-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 TBDMS-protected aldehyde 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, 9:1) to afford the title compound 19 as a pale yellow oil. Yield: 15 mg (50%).

**Biologically active compound:**

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, d5-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.33

**Preparation of Naturally Occurring PD1n3-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 cultured in DBPSHCOOH (5 × 105 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), PD1n3-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. 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), PD1n3-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, PD1n3-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 PD1n3-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 PD1n3-DPA (5) and related compounds. C.N.S.’s interests are reviewed and
ACKNOWLEDGMENTS

We are indebted to Dr. P. P. Molesworth, Department of Chemistry, University of Oslo, for skillful technical assistance with acquisition of 2D-NMR spectra. The Norwegian Research Council (KOSK II) and the School of Pharmacy, University of Oslo, are gratefully acknowledged for Ph.D. scholarships to M.A. and J.E.T., respectively. T.V.H. is grateful for a Leiv Eriksson travel grant from The Norwegian Research Council. J.D., R.C., and C.Y.C. are supported by the National Institutes of Health GM Grant PO1GM095467 (C.N.S.).

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