Synthesis of 13(R)-Hydroxy-7Z,10Z,13R,14E,16Z,19Z Docosapentaenoic Acid (13R-HDPA) and Its Biosynthetic Conversion to the 13-Series Resolvins

Karoline G. Primdahl,†,‡,§ Marius Aursnes,‡ Mary E. Walker,‡ Romain A. Colas,‡ Charles N. Serhan,⊥ Jesmond Dalli,*,‡ Trond V. Hansen,*,‡ and Anders Vik∗,‡

†School of Pharmacy, Department of Pharmaceutical Chemistry, University of Oslo, P.O. Box 1068 Blindern, N-0316 Oslo, Norway
‡William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, Charterhouse Square, London, UK, EC1M 6BQ
§Center for Experimental Therapeutics and Reperfusion Injury, Department of Anesthesiology, Perioperative and Pain Medicine, Harvard Institutes of Medicine, Brigham and Women’s Hospital and Harvard Medical School, Boston, Massachusetts 02115, United States

ABSTRACT: Specialized pro-resolving lipid mediators are biosynthesized during the resolution phase of acute inflammation from n-3 polyunsaturated fatty acids. Recently, the isolation and identification of the four novel mediators denoted 13-series resolvins, namely, RvT1 (1), RvT2 (2), RvT3 (3) and RvT4 (4), were reported, which showed potent bioactions characteristic for specialized pro-resolving lipid mediators. Herein, based on results from LC/MS-MS metabololipidomics and the stereoselective synthesis of 13(R)-hydroxy-7Z,10Z,13R,14E,16Z,19Z docosapentaenoic acid (13R-HDPA, 5), we provide direct evidence that the four novel mediators 1−4 are all biosynthesized from the pivotal intermediate 5. The UV and LC/MS-MS results from synthetic 13R-HDPA (5) matched those from endogenously and biosynthetically produced material obtained from in vivo infectious exudates, endothelial cells, and human recombinant COX-2 enzyme. Stereochemically pure 5 was obtained with the use of a chiral pool starting material that installed the configuration at the C-13 atom as R. Two stereoselective Z-Wittig reactions and two Z-selective reductions of internal alkynes afforded the geometrically pure alkene moieties in 5. Incubation of 5 with isolated human neutrophils gave all four RvTs. The results presented herein provide new knowledge on the biosynthetic pathways and the enzymatic origin of RvTs 1−4.

During the resolution of acute inflammation, a novel group of host-protective chemical mediators biosynthesized from the n-3 polyunsaturated fatty acids (PUFAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), termed specialized pro-resolving mediators (SPMs)† and their bioactive peptide-conjugates,‡ are resolution mediators and control tissue regeneration and promote the return to homeostasis.§ The resolvins, protectins, and maresins constitute individual families of SPMs that are formed via distinct biosynthetic pathways.† During the resolution of acute inflammation, SPMs exhibit a wide range of potent pro-resolving actions, which include promoting the clearance of bacteria and apoptotic cells, counter-regulating the production and actions of pro-inflammatory mediators, and stimulating the resolution of inflammation.¶ The PUFA n-3 docosapentaenoic acid (n-3 DPA) is an intermediate in the biosynthesis of DHA from EPA and is also a precursor to novel bioactive mediators.†† The isolation and structure elucidation of four new host-protective molecules was recently reported. These compounds were termed 13-series resolvins (RvTs), namely, RvT1 (1), RvT2 (2), RvT3 (3) and RvT4 (4), given that they share a hydroxy functionality at carbon 13.¶

These four enzymatically oxygenated products are biosynthesized during neutrophil-endothelial cocultures and are present, after sterile inflammation as well as infection, in both human and mouse tissues. These four new natural products are biosynthesized from n-3 DPA during neutrophil-endothelial interactions where endothelial cyclooxygenase-2 (COX-2) converts n-3 docosapentaenoic acid to 13-hydro(peroxy)-7Z,10Z,13R,14E,16Z,19Z docosapentaenoic acid that is then thought to react rapidly, via COX-2-mediated peroxidase activity, into 13(R)-hydroxy-7Z,10Z,13R,14E,16Z,19Z docosapentaenoic acid (Scheme 1).¶ The RvTs contain conjugated diene and triene moieties, as well as isolated Z-olefins, in

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As bacterial infections in humans remain a serious health concern due to the rise in antibiotic resistance toward existing antibacterial therapeutics, an imminent need for new treatment strategies exists. Of interest toward this aim, the RvTs 1–4 exert anti-inflammatory and potent pro-resolving activities by regulating key innate protective responses during *E. coli* infections in mice. Due to the interesting and potent biological activities of the RvTs 1–4, it is of considerable interest to further investigate their biosynthetic pathways. Herein, we present direct evidence for the configurational assignment of the key biosynthetic intermediate in the RvT pathway, namely, 13R-HDPA (5), by matching material obtained from total synthesis with that isolated from (a) human endothelial cells, (b) mouse infectious exudates, and (c) human recombinant COX-2. We also demonstrate that 13R-HDPA is converted by human neutrophils to all four RvTs, thereby confirming the role of 13R-HDPA as a key biosynthetic intermediate in RvT formation.

## RESULTS AND DISCUSSION

To establish evidence of the existence of 13R-HDPA (5) as a pivotal intermediate and its role in the biosynthesis of the novel 13-series resolvins (RvT 1–4), stereocorechemically pure 5 was obtained by total synthesis. First, a synthesis of the terminal alkyne 10 from commercially available 2-pentyn-1-ol (6), Scheme 2, was needed. The preparation of diyne 8 was performed as previously reported. Unfortunately, attempted Lindlar reduction of the internal alkyne in 8 gave no conversion to wanted 9. However, the stereoselective Z-reduction of the internal alkyne was successfully achieved with P-2 nickel boride (P-2 Ni), which provided 9 in 80% yield. Removal of the TMS-protecting group was achieved using TBAF buffered with acetic acid. Addition of acetic acid was absolutely necessary to suppress the formation of the E-isomer of 10. The modest overall yield of 10 by this sequence is attributed to the high volatility of 10.

Next, vinyl iodide 13 was prepared from commercially available (R)-α-hydroxy-γ-butyrolactone (11), via known alcohol 12. An alkyne hydrozirconation of 12, followed by treatment with iodine, furnished the vinyl iodide 13 in fair yield (Scheme 3).

The Wittig salt 19 was prepared from cycloheptanone (14) in eight steps. Phosphonium salt 15 was obtained as previously reported in a sequence involving a Baeyer–Villiger oxidation of 14, subsequent methanalysis of the resulting lactone,
conversion of the formed primary alcohol to its iodide that was reacted with triphenylphosphine to give 15. A Wittig reaction of 15 with TBS-protected 3-hydroxypropanal (16) and subsequent removal of the silyl group15 afforded the alcohol 17. This was then converted into the corresponding iodide 18 via an Appel reaction, followed by treatment with triphenylphosphine in refluxing acetonitrile that afforded the desired Wittig salt 19 in 81% yield over the two steps (Scheme 4).

Then terminal alkyne 10 and vinyl iodide 13 were assembled in a Sonogashira cross-coupling reaction, which produced the conjugated enyne 20 in 86% yield. Oxidation of the alcohol in 20 gave aldehyde 21, which was immediately reacted in a Z-selective Wittig reaction with the ylide of Wittig salt 19; the latter was generated at −78 °C after treatment with NaHMDS. This afforded the ester 22 in 77% yield. Synthetic 13R-HDPA (5) also gave R<sub>T</sub> 5.1 min (Figure 2D), and when this material was coinjected with biological material, a sharp peak only at R<sub>T</sub> 5.1 min was observed (Figure 2E). Of note, in the endothelial cell and recombinant enzyme incubations, we also identified the S-isomer of 13-HDPA that was found to be the minor product in both incubations forming ~10% of the overall 13-HDPA levels in the HUVEC incubations (Figure 2A) and <5% in the recombinant enzyme incubations (Figure 2C). This is in accordance with published findings that COX-2 stereoselectively converts n-3 DPA to the R-configured stereoisomer with a small proportion of the substrate being converted to the S-stereoisomer.8 This is also observed for other enzymes that lipoxygenate their substrate.19 Altogether, these efforts established that the synthetic material of 5 eluted together with biologically produced 13-HDPA and that the absolute configuration at C-13 is R for biogenic 13-HDPA (5).

To obtain further evidence for matching physical properties of authentic and synthetic material of 5, MS-MS spectra for 13R-HDPA from HUVEC incubations, infectious exudates and synthetic material of 5 were recorded, that gave essentially identical MS-MS spectra including fragments at m/z 327, 301, 283, 223, 205, and 195 (Figure 3A–C).
Figure 1. 13-HDPA MRM chromatograms from (A) endothelial cells, (B) infectious exudates, (C) hrCOX-2, and (D) synthetic material of 5. (E) Coinjection of endothelial and synthetic 5.

Figure 2. 13-HDPA chiral LC-MS-MS derived from (A) endothelial cells, (B) infectious exudates, (C) hrCOX-2, and (D) synthetic material. (E) Coinjection of endothelial and synthetic 13R-HDPA.
Assessment of the UV chromophore of biogenic 13-HDPA from hrCOX-2 and that of the synthetic material of S, both gave $\lambda_{\text{max}}^{\text{MeOH}}$ of 237 nm (Figure 4A,B), adding additional confidence in our structural assignment.

We next tested whether the synthetic material S was a substrate for the conversion to any of the four RvTs by human neutrophils. Incubation of synthetic 13R-HDPA (S) with human neutrophils gave RvT1 (1), RvT2 (2), RvT3 (3), and RvT4 (4), as determined using both retention time (Figure 5A) and MS-MS spectra (Figure 5B). Of note in incubations with neutrophils, the synthetic material S was not converted to any of the four RvTs by human neutrophils. Incubation of synthetic 13R-HDPA (S) with human neutrophils gave RvT1 (1), RvT2 (2), RvT3 (3), and RvT4 (4), were >75% lower than those found in incubations with the synthetic material. These results are in accordance with published findings and indicate that while PMN may utilize endogenous 13R-HDPA, which may be esterified and released upon cellular activation as observed for other SPM biosynthetic intermediates, these cells rely on other cell types to donate this key biosynthetic intermediate for RvT biosynthesis. Together these results establish the exact structural assignment of 13R-HDPA as 13(R)-hydroxy-7Z,10Z,13R,14E,16Z,19Z-docosapentaenoic acid (S), as well as its key role in the RvT biosynthetic pathway.

**CONCLUSIONS**

The biosynthesis of SPMs in human physiological systems affords the E- and D-series resolvins with either a E,E,Z,E-tetraene or a E,E,Z-triene moiety. On the other hand, the 13R-series-resolvins display diene and triene moieties, isolated Z-olefins, and a hydroxy functionality at C-13. These features distinguish the 13R-series-resolvins from the established families of SPMs (i.e., the resolvins, protectins, and maresins), as well as other oxygenated natural products of nonhuman origin. Herein, we have demonstrated that the COX-2 enzyme is involved in the first step in the biosynthetic pathways of the 13R-series-resolvins. Most likely, as for the other SPM families, the first step involves the formation of a hydroperoxide intermediate that undergoes distinct enzymatic multistep sequences to the individual natural products 1–4. Because all families of SPMs, as well as other oxygenated PUFA-derivatives, exhibit potent and interesting pharmacological actions, these natural products are of interest as lead compounds toward the clinical development of different treatments for human diseases, via a novel mechanism as resolution agonists. Such efforts will be more expedient with knowledge of the complete structural assignment and biosynthetic pathways of SPMs such as the 13R-series resolvins.

**EXPERIMENTAL SECTION**

General Experimental Procedures. Optical rotations were measured using a 0.7 mL cell with a 1.0 dm path length on an Anton Paar MCP 100 polarimeter. The UV–vis spectra from 190 to 900 nm were recorded on a Shimadzu UV-1800 spectrophotometer using quartz cuvettes. NMR spectra were recorded on a Bruker AVIII400 spectrometer at 400 MHz or a Bruker AVIII600 spectrometer at 600 MHz for $^1$H NMR, and at 100 or 150 MHz for $^{13}$C NMR. Spectra are referenced relative to the central residual protium solvent resonance in $^1$H NMR (CDCl$_3$ δ$_{H}$ = 7.27, and MeOH-d$_4$ δ$_{H}$ = 3.31) and the central carbon solvent resonance in $^{13}$C NMR (CDCl$_3$ δ$_{C}$ = 77.00 ppm, and MeOH-d$_4$ = δ$_{C}$ = 49.00). High-resolution mass spectra were recorded on a Waters Prospec Q spectrometer using 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 F254 aluminum-backed plates fabricated by Merck. HPLC analyses for chemical purities were performed on an Agilent Technologies 1200 Series instrument with diode array detector set at 254 nm and equipped with a C18 stationary phase.

Figure 3. MS-MS spectra employed in the identification of 13-HDPA from (A) endothelial cells, (B) infectious exudates, and (C) synthetic material. n = 3 endothelial cell donors, n = 3 mouse exudates, and n = 3 for synthetic material.
(Z)-Hept-4-en-1-yn-1-yltrimethylsilane (9). A suspension of sodium borohydride (32.9 mg, 0.87 mmol) in EtOH (1.3 mL) was added dropwise to a flask containing nickel acetate tetrahydrate (217 mg, 0.87 mmol, 14 mol%) in EtOH (13.0 mL) at 0 °C under stirring. The reaction mixture turned black. After stirring for 15 min at room temperature, ethylenediamine (116 μL, 105 mg, 1.74 mmol) was added, and the stirring was continued for 10 min. The flask was evacuated and refilled with hydrogen gas before the skipped diyne 8 was added (1.00 g, 6.09 mmol, 1.00 equiv). The reaction mixture was stirred at room temperature under hydrogen atmosphere until completion (~4 h), then filtered through a short plug of silica gel that was washed with pentane (10 mL). The filtrate was transferred to a separatory funnel and washed with H2O (3 × 5.0 mL). The organic layer was dried (MgSO4), and the solvent was removed in vacuo.

Figure 4. UV spectra for (A) hrCOX-2 13-HDPA and (B) synthetic 13R-HDPA (5).

Figure 5. Human neutrophils convert 13R-HDPA (5) to RvT1−4 (1−4). Human neutrophils were isolated from peripheral blood of healthy donors and incubated (2 × 10⁷ cells/mL) with or without 13R-HDPA (5) (45 min, 37 °C, 2 μM A23187, PBS, pH = 7.45). Incubations were quenched with two volumes of ice cold MeOH and products extracted and profiled using lipid mediator metabololipidomics. (A) MRM chromatograms for each of the RvT1−4 with relative abundances to their levels in each of the incubations. (B) MS-MS spectra employed in the identification of RvT1 (1), RvT2 (2), RvT3 (3) and RvT4 (4). Results are representative of n = 3 healthy volunteers.
Purification by flash chromatography on silica gel (pentane) afforded the desired product 9 as a colorless oil. Yield: 810 mg (80%); 1H NMR (400 MHz, CDCl3) δH 5.51–5.42 (m, 1H), 5.42–5.35 (m, 1H), 2.98 (m, 2H), 2.06 (p, J = 7.5 Hz, 2H), 0.98 (t, J = 7.5 Hz, 3H), 0.15 (s, 9H). 13C NMR (101 MHz, CDCl3) δC 137.3, 123.4, 103.5, 94.7, 92.4, 20.7, 18.4, 14.1, 0.3. TLC (hexane, KMN0 stain) Rf = 0.12. The spectroscopic data were in agreement with those previously reported for this compound.24

(Z)-Hept-4-ene-1-yne (10). (Z)-Hept-4-ene-1-yn-1-yltrimethylsilane (9) (700 mg, 4.21 mmol, 1.00 equiv) was added to a solution of TBAF (1.90 (q, J = 6.8 Hz, 2H), 1.52 (p, J = 7.4 Hz, 2H), 1.36–1.19 (m, 4H). 1H NMR (101 MHz, MeOH-δ) δH 7.48 (2H), 7.46 (2H), 7.36 (2H), 5.21 (s, 3H), J′ = 16.1 Hz, 3C), 52.0, 46.9, 39.4, 8.00 (s, 3H), 0.15 (s, 3H); 13C NMR (101 MHz, CDCl3) δC 175.8, 136.3 (d, 4C), 127.4 (m, 1H), 123.5 (m, 1H), 119.8 (s, 1H), 119.8 (d, 1C), 61.2, 59.5, 52.0, 39.4, 25.9 (3C), 18.3, –4.4, –4.9; HRESITOFMS: m/z 365.0403 [M + Na]+ (calcd for C17H15O2SiNa, 331.2064); TLC (hexane/EtOAc 7:3, KMN0 stain) Rf = 0.37.

(R,E)-3-((tert-Butyldimethylsilyl)oxy)-5-iodopent-4-en-1-ol (11). 2-Chloro-2-methylpropanol (78 mL, 1.23 equiv) was added in one portion, and the reaction mixture was stirred for 30 min at 0 °C before it was allowed to slowly warm up to room temperature and stirred for 1 h. The reaction was quenched with saturated aq NaHCO3 (33.3 ml), extracted with CH2Cl2 (2 × 15.0 ml), dried (Na2SO4), and concentrated in vacuo. The crude product was purified by column chromatography on silica gel (heptane/EtOAc 7:3) to afford hydroxyl methyl ester 17 as a clear oil. The chemical purity (>99%) was determined by GLC analysis: Initial temperature 100 °C, rate: 5°C/min, final temperature 200 °C, t1 (minor) = 8.90 min, t2 (major) = 9.22 min. Yield: 483 mg (76%); 1H NMR (400 MHz, CDCl3) δH 7.08–6.90 (m, 2H), 5.21 (s, 3H), 5.09 (t, J = 7.0 Hz, 2H), 3.92–3.77 (m, 4H), 3.11–3.02 (m, 2H), 2.61 (dd, J = 16.0, 6.7 Hz, 2H, 1H), 2.52 (dd, J = 16.0, 5.1, 2.1 Hz, 1H), 2.07 (p, J = 7.3 Hz, 2H), 0.98 (t, J = 7.5 Hz, 3H), 0.87 (s, 3H), 0.06 (s, 3H), 0.05 (s, 3H). 13C NMR (101 MHz, CDCl3) δC 201.1, 143.1, 133.8, 123.3, 110.7, 90.1, 77.9, 68.6,
SIL 3.5, 25.9, 20.7, 18.2, 17.9, 14.1, −4.3, −4.9. HRESITOFMS: m/z 329.1907 [M + Na]⁺ (calcd for C₂₂H₃₄O₃Na, 329.1913); TLC (hexane/EtOAc 85:15, KMN0 stain) Rᵣ = 0.38.

Methyl-[(R,Z)-10Z,14E,19Z]-3-(3-[tert-butyl(dimethyl)siloxy])oxy)-docosa-7,10,14,19-tetraen-16-yne (22). Wittig salt 19 (374 mg, 65.4 μmol, 1.00 equiv) was dissolved in THF (8.80 mL) and HMPA (0.88 mL), cooled to −78 °C and added NaHMDS (0.6 M in toluene, 1.09 mL, 65.4 μmol, 1.00 equiv). The reaction mixture was stirred for 45 min before alkylation 21 (200 mg, 65.5 μmol, 1.00 equiv) in THF (0.88 mL) was added dropwise. The reaction was stirred for 1 h at −78 °C and then the reaction mixture was allowed to slowly warm to 0 °C. Phosphate buffer (4.7 mL, pH = 7.2) was added to quench the reaction and the aqueous phase was extracted with Et₂O (3 × 6.00 mL), and then the wet activated Zn was added quickly under a blanket of argon. The reaction was stirred for 10 h in the dark. After completion, the mixture was filtered through Celite with Et₂O and the aqueous phase was extracted with EtOAc (3 × 3.0 mL). The combined organic layers were dried (Na₂SO₄) and concentrated in vacuo. The crude product was purified by column chromatography on silica gel (heptane/EtOAc 95:5, KMnO₄ stain) to afford compound 22 as a pale yellow oil. Yield: 239 mg (77%); [α]D₂₀ + 2.1 (c 0.39, CHCl₃).

1H NMR (400 MHz, MeOH-d₄) δ 5.98 (dd, J = 15.8, 5.8 Hz, 1H), 5.62 (dq, J = 15.8, 2.0 Hz, 1H), 5.51–5.29 (m, 6H, 4.22) (c = 5.6 Hz, 2H), 3.05 (s, 3H). The reaction mixture was neutralized with a 10% aq solution of NaHCO₃ (140 μL of 6 M NaHCO₃) and then stirred under argon at 0 °C for 30 min at room temperature. Incubations were stopped with two volumes of MeOH containing deuterium oxide. HPLC analysis (Eclipse XDB-C18, MeOH/H₂O 85:15, 1.0 mL/min): r (major) = 17.53 min, and r (minor) = 13.72 and 17.53 min.

Biogenic 13R-HDPA. 13R-HDPA (5). Methyl ester 24 (12.0 mg, 33.3 μmol, 1.00 equiv) was dissolved in THF/MeOH/H₂O (2:2:1, 3.90 mL) and cooled to 0 °C. LiOH (24 mg, 0.6 mmol, 30.0 equiv) was added in one portion. The reaction mixture was stirred for 3 h at 0 °C before it was allowed to warm up to room temperature and stirred until completion, as monitored by TLC (t < 2 h). Saturated aq NaH₂PO₄ (60.0 mL) was added. The aq phase was extracted (2 × 3.0 mL) dried (Na₂SO₄), and the solvent was removed in vacuo. The crude product was purified by column chromatography on silica gel (heptane/EtOAc 50:50, KMnO₄ stain) to afford the hydroxy acid 5 as colorless oil. Yield: 10.0 mg (87%); [α]D₂₀ − 0.64 (c 0.47, MeOH); UV(MeOH) λmax 236 (log ε = 4.39); 1H NMR (400 MHz, MeOH-d₄) δ 6.55 (dd, J = 15.2, 11.2 Hz, 1H), 5.97 (t, J = 10.9 Hz, 1H), 5.68 (dd, J = 15.2, 6.5 Hz, 1H), 5.50–5.26 (m, 7H), 4.15 (q, J = 6.5 Hz, 1H), 2.94 (t, J = 7.2 Hz, 2H), 2.80 (t, J = 5.8 Hz, 2H), 2.40–2.22 (m, 4H), 2.09 (q, J = 7.6, 6.9 Hz, 1H), 1.61 (p, J = 7.4 Hz, 2H), 1.38 (m, 4H), 0.98 (t, J = 7.5 Hz, 3H); 13C NMR (101 MHz, MeOH-d₄) δc 177.7, 137.1, 133.1, 131.2, 131.1, 130.9, 129.2, 129.0, 127.8, 126.4, 73.1, 52.0, 34.8, 30.4, 29.8, 28.0, 26.8, 25.9, 21.5, 14.6; HRESITOFMS: m/z 383.2555 [M + Na]⁺ (calcd for C₂₃H₃₄O₃Na, 383.2562); TLC (hexane/EtOAc 75:25, KMnO₄ stain) Rᵣ = 0.33. The chemical purity (>98%) was determined by HPLC analysis (Eclipse XDB-C18, MeOH/H₂O 85:15, 1.0 mL/min): tᵣ (minor) = 13.72-17.53 min, and tᵣ (major) = 16.53 min. 8

Human umbilical vein endothelial cells (HUVEC; 8.5 × 10⁶ cells/9.6 cm²) were incubated with IL-1α (10 ng/mL) and TNF-α (10 ng/mL; 16 h, 37 °C, 5% CO₂). Incubations were quenched using 2 volumes of MeOH containing deuterium oxide labeled d₅-SS-HETE. In separate experiments n-3 DPA (Cayman Chemical Company) was incubated with human recombinant COX-2 (Cayman Chemicals; in 0.1 M Tris·HCl, pH 8.0; 20 μM porcine hematin, 0.67 mM phenol) for 30 min at room temperature. Incubations were stopped with two volumes of MeOH and products extracted using diethyl ether. 13-HDPA was isolated using RP-HPLC (1260 Series; Agilent Technologies) and an Agilent C18 Poroshell column (3.5 μm, 4.6 × 25 cm) with a mobile phase consisting of MeOH/H₂O (60:40, vol/vol) at 0.5 mL/min that was ramped up to 98:2 (v/v) for 20 min.

Infectious exudates were collected from mice (6–8 weeks old, male, FvB, Charles River, UK) 12 h after administration of E. coli (10⁵ CFU). Exudates were collected in 4 mL of PBS (containing calcium and magnesium) and placed in 2 volumes of ice-cold MeOH containing d₅-SS-HETE and commercially available lipid mediators. In these experiments, male FvB mice (6–8 weeks of age) were used. These animals were maintained on a standard chow pellet diet and had access to water ad libitum, with a 12-h light–dark cycle. All animal experiments were approved and performed under the guidelines of the Ethical Committee for the Use of Animals, Barts and The London School of Medicine, and in accordance with the UK Home Office regulations (Guidance on the Operation of Animals, Scientific Procedures Act, 1986).

RVT Biosynthesis. Human peripheral blood was collected according to a protocol approved by Barts and the London Research Ethics Committee, London, United Kingdom (QMREC 2014:61). Written informed consent was received from participants prior to inclusion in...
the study according to the Declaration of Helsinki. Neutrophils were prepared following density separation by layering on Ficoll-Histopaque 1077−1. The cells were then centrifuged at 300g (30 min, 4 °C), and contaminating red blood cells were lysed by hypotonic lyses as in ref 8. Neutrophils 20 × 10^6 cells/mL (PBS−, pHi = 7.45) were then incubated with synthetic 13R-HDPA (5) (10 μM) for 45 min (37 °C). Incubations were stopped with 2 volumes of ice-cold MeOH. NaBH₄ was then added to reduce the hydroperoxides produced by the neutrophil lipoxigenases (1.0 mg/ml; Sigma-Aldrich), and products were isolated using C18 solid phase extraction as detailed below.

**Lipid Mediator Profiling.** MeOH (two volumes) was added to cell incubations, plasma (mouse and human), and infectious exudates, and samples were stored at −20 °C until extraction. Prior to extraction, samples were then centrifuged (1200g, 4 °C, 10 min). Supernatants were then collected and brought to less than 1.0 mL of MeOH content. Neutrophils 20 × 10⁵ were isolated using C18 solid phase extraction as detailed below. Neutrophils 20 × 10⁵ (Biotage). The RvT1−4 and 13-HDPA products were extracted using an ExtraHerA (Biotage) automated extraction system as follows. Solid-phase C18 cartridges were equilibrated with 3.0 mL of MeOH and 6.0 mL of H₂O. Nine milliliters of aq HCl solution (pH = 3.5) was then added to the samples, and the acidified solutions were rapidly loaded onto the conditioned C18 columns that were washed with 4.0 mL of H₂O. Next, 5.0 mL of hexane was added, and the products were eluted with 4.0 mL of methyl formate. Products were brought to dryness using the automated evaporation system (TurboVap LV system, Biotage) and immediately suspended in MeOH−H₂O (50:50 vol/vol) for LC-MS-MS automated injections as previously reported.¹

Extracted samples were analyzed by an LC-MS-MS system, Qtrap 5500 (AB Sciex) equipped with a Shimadzu SIL-20AC autoinjector and LC-20AD binary pump (Shimadzu Corp.). A Poroshell C18 column (100 mm × 4.6 mm × 2.7 μm) was used with a gradient of MeOH/H₂O:AcOH of 55:45:3.5:0.01 (v/v/v/v) that was ramped to 85:15:0.01 (v/v/v/v) over 10 min and then to 98:2.00:0.01 (v/v/v/v) for the next 8 min. This was subsequently maintained at 98:2.00:0.01 (v/v/v/v) for 2 min. The flow rate was maintained at 0.4 mL per minute. To monitor and quantify the levels of lipid mediators, a multiple reaction monitoring (MRM) method was developed with signature ion fragments (m/z) for each molecule monitoring the parent ion (Q1) and a characteristic daughter ion (Q3). Identification was conducted using published criteria where a minimum of 6 diagnostic ions were employed, see ref 18 for details. Detection limit was ~0.1 pg.

For chiral-phase lipidomic analysis, a Chiralpak AD-RH column (150 mm × 2.1 mm × 5 μm) was used with isocratic MeOH/H₂O:AcOH 95:5.01:0.01 (v/v/v) at 0.15 mL/min. To monitor isobaric monohydroxy docosapentaenoic acid levels, a multiple reaction monitoring (MRM) method was developed using signature ion fragments 345 > 195 described.²

**ASSOCIATED CONTENT**

1 Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.6b00634.

¹H, ¹³C spectral data, HRMS and UV−vis spectra, as well as chromatograms from HPLC analyses of synthetic intermediates and 13R-HDPA (5) (PDF)

**AUTHOR INFORMATION**

**Corresponding Authors**

*E-mail: j.dalli@qmul.ac.uk.

*E-mail: tv.hansen@farmasi.uio.no.

*E-mail: anders.vik@farmasi.uio.no.

**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 RvT1 (1), RvT2 (2), RvT3 (3), RvT4 (4), 13R-HDPA (5) and related compounds. C. N. Ss interests are reviewed and are managed by BWH and Partners HealthCare in accordance with their conflict of interest policies.

³(K.G.P.) On leave from the School of Pharmacy, Department of Pharmaceutical Chemistry, University of Oslo.

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