The First Total Synthesis of the Lipid Mediator PD2n-3 DPA

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ABSTRACT: The resolution of inflammation is governed by the active biosynthesis of specialized pro-resolving mediators using ω-6 and ω-3 polyunsaturated fatty acids as substrates. These mediators act as resolution agonists and display several interesting bioactivities. PD2n-3 DPA is an oxygenated polyunsaturated fatty acid biosynthesized from n-3 docosapentaenoic acid belonging to the specialized pro-resolving lipid mediator family named protectins. The protectins exhibit anti-inflammatory and pro-resolving bioactivities. These endogenously produced compounds are of interest as leads in resolution pharmacology and drug development. Herein, together with its NMR, MS, and UV data, a stereoselective total synthesis of PD2n-3 DPA is presented.

Endogenous mechanisms that control resolution programs during acute inflammation are essential in maintaining health.1 If uncontrolled, chronic inflammation may result in the development of several human diseases.1,2 Individual families of specialized pro-resolving mediators (SPMs) are the lipoxins, the resolvins, the maresins, and the protectins. SPMs are oxygenated polyunsaturated fatty acids (PUFAs) that stimulate the resolution of inflammation and fight infections.5 The ω-3 PUFAs eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and n-3 docosapentaenoic acid (n-3 DPA) are, in the presence of cyclooxygenase-2 and various lipoxigenases, converted into SPMs.3 SPMs exhibit anti-inflammatory and pro-resolving bioactions in the low molar range due to their agonist effect on G-protein coupled receptors (GPCRs).4 Such resolution processes have attracted interest in drug development research programs, given that approximately 30% of all approved drugs act on this receptor family.4 Because SPMs are endogenously biosynthesized in minute amounts, stereoselective total synthesis becomes necessary for configurational assignment and extensive biological evaluations facilitating drug development efforts.4

In 2013 and 2015, new SPMs biosynthesized from n-3 DPA were reported.6 N-3 DPA is a biochemical intermediate in the formation of DHA from EPA.7 Some examples of n-3 DPA derived SPMs are shown in Figure 1.6a

Stereoactive total synthesis of 1 confirmed its structure as shown in Figure 1.b In collaboration with the Dalli group, we elucidated the biosynthetic pathway of PD1alpha3 DPA (1) and PD2alpha3 DPA (2) as depicted in Scheme 1.10 Oxidation of n-3 DPA by 15-LOX gives the hydroperoxide S, which is transformed into the epoxide ePDalpha3 DPA (6). Hydrolysis of 6 in the presence of an unknown enzyme provides 1 and 2.10

The SPM 1 displayed potent anti-inflammatory and pro-resolving bioactions together with stimulation of human macrophage phagocytosis and effectorcytosis.6 In the resolution of inflammation these bioactions are distinct features.2–4 As of today, no total synthesis of PD2alpha3 DPA (2) has been reported. Against this, and the interesting bioactions of the n-3 DPA derived SPMs,11 the first total synthesis of 2 is presented.

RESULTS AND DISCUSSION

Based on the biosynthesis presented in Scheme 1, we anticipate the diol moiety in PD2alpha3 DPA (2) to be anti and 16R,17S configured. Hence, our synthesis of 2 was planned as outlined in Scheme 2, leading back to the triene-aldehyde 7 and the known Wittig salt 8; the latter has been prepared from 11 and 12.12 Compound 9 may be prepared from the Wittig reagent 13 and 2-deoxy-D-ribose (14), both commercially available.

Aldehyde 15 was prepared from 2-deoxy-D-ribose (14) over three steps as previously reported14 (Scheme 3). Aldehyde 15 was reacted with the ylide of commercially available triphenyl-

PD2n-3 DPA
(propyl)phosphonium bromide (13) in a Z-selective Wittig reaction. The ylide of 13 was formed after reaction with NaHMDS in CH$_2$Cl$_2$ at −78 °C. After column chromatography, 16 was obtained as one stereoisomer in 86% yield.

Next, we used our monodeprotection protocol$^{11c}$ in order to obtain alcohol 17, which was oxidized to the aldehyde 9. Reacting 9 with an excess of 10 afforded the desired aldehyde 7 in 28% isolated yield over the three steps. The desired product was readily separated from substantial quantities of the mono Wittig product also produced, by column chromatography. Of notice, ylide 10 had to be added in several portions over the course of the reaction due to the instability of 10 at elevated temperatures.

Next the ylide of known Wittig-salt 8 was generated (NaHMDS, THF, HMPA, −78 °C) and reacted with commercially available aldehyde 11. This yielded compound 18, which was converted (camphorsulfonic acid (CSA), MeOH, CH$_2$Cl$_2$) into alcohol 19. Alcohol 19 was isolated in 70% yield from 11. The Wittig-salt 8 was obtained in 87% yield from 19 over two steps (I$_2$, PPh$_3$, imidazole; PPh$_3$). Again,
NaHMDS in THF and HMPA at ~78 °C was used in a Z-selective Wittig reaction, this time with 8 and aldehyde 7, affording 20 in 47% yield (Scheme 4). Deprotection of the two methylene carbons at 27.1 and 31.8 ppm, next to the C-19(reporting), allowed the structural characterization of a triene chromophore that is selective Wittig reaction, this time with 8 NaHMDS in THF and HMPA at

**Scheme 4. Synthesis of PD2n-Z DPA (2)**

NaHMDS, THF, HMPA, 78 °C

1. NaHMDS, THF, HMPA, 78 °C

2. Aldehyde 7, 47%

TBS-ethers was achieved with TBAF, yielding the methyl ester 21 in 74% yield and with >96% chemical purity (HPLC, Supporting Information); see Scheme 4. Basic hydrolysis gave PD2n-Z DPA (2) in 91% yield.

The assignment of the Z- or E-configuration for each of the double bonds was then performed using two-dimensional NMR spectroscopy. From these experiments, connectivity between the adjacent olefinic protons (H-7/H-8, H-10/H-15, and H-19/H-20) was observed (Table 1, Scheme 1). Combining these analyses with the data from the HMBC spectra allowed the assignment of all the olefinic hydrogens. The signals at 5.83 ppm (dd, 1H, J = 15.0, 7.1 Hz) and 6.26 ppm (dd, 1H, J = 14.6, 10.7 Hz) are diagnostic for E-double bonds, while the signal observed at 6.03 ppm (apparent t, 1H, J = 11.1 Hz) correlates with a Z-double bond.

The COSY spectrum was used for assigning vicinal signals for each of the two isolated Z-olefins and the Z,E,E-moity. Moreover, two signals from the hydrogen atoms attached to the carbinol carbon atoms were observed as expected with signals at 4.01 ppm (ddd, 1H, J = 7.0, 4.9, 1.1 Hz) and 3.55 ppm (dt, 1H, J = 8.1, 4.9 Hz). MaR2n-Z DPA (3) and RvD2n-3 DPA (4) displayed similar chemical shift values and coupling pattern. Of note, these SPMs were matched against authentic materials using LC/MS-MS analysis.

The HSQC spectrum was used for assigning the signals from the methyl carbons at 27.1 and 31.8 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).

Moreover, the ultraviolet absorbance profile of 2 gave absorbance characteristics of a triene chromophore that is allylic to an auxochrome with λmax (MeOH) = 272 nm with shoulders at 262 and 283 nm.

The use of 2-deoxy-D-ribose (14) as a chiral pool starting material enabled the introduction of the 16R,17S-configured diol moiety in PD2n-Z DPA (2). This sugar was used by Rodriguez and Spur in their synthesis of PD2, the 4,5-Z double-bond congener of 11a. The 17S configuration is biosynthetically formed by a stereoselective oxygenation of n-3 DPA by the 15-LOX enzyme (Scheme 2). The COX-2 enzyme produces an R-configured hydroperoxide intermediate with DHA, n-3 DPA, or 3-oxa n-3 DPA as substrate. It has been reported that for anti-1,2-diol-containing protectins and resolvins, also biosynthesized via epoxide intermediates, an Sn2-type opening by water takes place at the activated allylic carbon atom of the epoxide. This process occurs both for epoxides formed via the 15-LOX or the COX-2 pathways.

### Table 1. Compilation of 1H and 13C NMR Data of PD2n-Z DPA (2)

| Position | δC,a | δH,b | J (Hz) | HMBC | COSY |
|----------|------|------|--------|------|------|
| 1 | 177.8, C | | | | |
| 2 | 35.1, CH2 | 2.28, t (7.5) | | | |
| 3 | 26.1, CH2 | 1.62, quint (7.3) | | | |
| 4 | 29.9, CH2 | 1.39, m | | | |
| 5 | 30.4, CH2 | 1.39, m | | | |
| 6 | 28.0, CH2 | 2.11, m | | | |
| 7 | 131.5, CH | 5.41, m | | | |
| 8 | 128.5, CH | 5.38, m | | | |
| 9 | 27.1, CH2 | 2.96, t (7.3) | | | |
| 10 | 131.3, CH | 5.40, m | | | |
| 11 | 129.6, CH | 6.03, apparent t (11.1) | | | |
| 12 | 129.1, CH | 6.57, dd (14.3, 11.2) | | | |
| 13 | 133.7, CH | 6.26, dd (14.6, 10.7) | | | |
| 14 | 133.6, CH | 6.36, ddd (15.1, 10.7, 1.1) | | | |
| 15 | 133.8, CH | 5.83, dd (15.0, 7.1) | | | |
| 16 | 76.3, CH | 4.01, ddd (7.0, 4.9, 1.1) | | | |
| 17 | 76.0, CH | 3.55, dt (8.1, 4.9) | | | |
| 18 | 31.8, CH2 | 2.33 + 2.16, m | | | |
| 19 | 126.3, CH | 5.45, m | | | |
| 20 | 134.4, CH | 5.46, m | | | |
| 21 | 21.7, CH2 | 2.08, m | | | |
| 22 | 14.6, CH | 0.97, t (7.5) | | | |

**a**Measured at 100 MHz. **b**Measured at 400 MHz. **c**HMBC correlations are from proton(s) stated to the indicated carbon(s). The ppm values listed above for δC were assigned using the center of the COSY- and HSQC-peak intensities.
CONCLUSIONS

To conclude, the first total synthesis of the oxygenated PUFA product PD2,3-DPA (2) is reported, enabling its exact structural assignment. The key synthetic reactions were E- and Z-selective Wittig reactions, avoiding the use of challenging Z-selective reduction protocols of internal alkynes. Multimilliograms of material are now available for biological testing.

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 using an Agilent Technologies Cary 4845 UV–vis spectrophotometer using quartz cuvettes. NMR spectra were recorded on a Bruker Avii 400 or a Bruker Avii HD 400 spectrometer at 400 MHz or a Bruker AVIII600 spectrometer at 600 MHz for 1H NMR and at 100 or 150 MHz for 13C NMR. Spectra are referenced relative to the central residual proton solvent resonance in 1H NMR (CDCl3, δ 7.26, DMSO-d6, δ 2.50 and methanol-d4, δ 3.31) and the central carbon solvent resonance in 13C NMR (CDCl3, δ 77.00, DMSO-d6, δ 39.43 and methanol-d4, δ 49.00). Mass spectra were recorded at 70 eV on a Waters Prospec Q or Micromass QTOF 2W spectrometer using ESI as the method of ionization. High-resolution mass spectra were recorded at 70 eV on a Waters Prospec Q or Micromass QTOF 2W spectrometer using ESI as the method of ionization.

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aqueous phase was extracted with EtO₂ (2 × 15 mL), and the combined organic layers were dried (Na₂SO₄) before being concentrated in vacuo. The title compound 20 (65 mg, 0.11 mmol, 47%) was obtained after purification by column chromatography (hexane/EtOAc, 95:5; as an orange oil: R₂ (hexane/EtOAc, 95:5) = 0.22; [α]²⁰° +13 (c 0.5, MeOH); ¹H NMR (400 MHz, CDCl₃) δ 6.50–6.41 (m, 1H), 6.27–6.10 (m, 2H), 6.03 (apparent t, J = 10.9, 1.7 Hz, 1H), 5.68 (dd, J = 14.5, 7.5 Hz, 1H), 5.50–5.28 (m, 5H), 4.02 (dd, J = 7.5, 4.1 Hz, 1H), 3.66 (s, 3H), 3.62 (m, 1H), 2.93 (t, J = 6.9 Hz, 2H), 2.31 (t, J = 7.5 Hz, 2H), 2.27–2.16 (m, 2H), 2.12–1.97 (m, 4H), 1.63 (p, J = 7.4 Hz, 2H), 1.49–1.20 (m, 4H), 0.95 (t, J = 7.5 Hz, 3H), 0.87 (18H), 0.49–0.94 (m, 12H); ¹³C NMR (100 MHz, CDCl₃) δ 174.3, 134.6, 133.3, 132.8, 131.8, 130.5, 128.8, 127.6, 127.5, 125.5, 77.1, 76.9, 51.6, 34.2, 31.8, 29.4, 28.9, 27.2, 26.3, 26.1, 26.1, 25.0, 20.9, 18.4, 18.3, 14.3, –3.9, –4.0, –4.3, –4.5; HRESIMS m/z 627.4234 [M + Na]⁺ (calcd for C₉₂H₁₄₀O₄Na, 385.2349).

Methyl (7Z,10Z,12E,14E,16β,17S,19R)-16,17-Dihydroxydocosa-7,10,12,14,19-pentaenoate (21). A solution of TBS-protected diol 20 (32.5 mg, 53.7 µmol, 1.00 equiv) in THF (1.5 mL) was cooled to 0 °C, and TBAF (1.0 M in THF, 0.161 mL, 0.161 mmol, 3.00 equiv) was added. The reaction was stirred for 16 h before it was quenched with phosphate buffer (pH = 7.0, 0.5 mL). Brine (1.0 mL) and EtOAc (1.0 mL) were added, and the phases were separated. The water phase was extracted with EtOAc (2 × 1.0 mL). The combined organic phases were dried (Na₂SO₄) and concentrated in vacuo. The crude product was purified by column chromatography (SiO₂ hexane/EtOAc, 60:40) to afford the methyl ester of PD₂n-3_DPA (21) as a mixture of C-10/C-11-isomers (Z/E, 17:3). The Z-C-10/C-11 isomer 21 was obtained (15 mg, 39.8 µmol, 74%) as a pale yellow oil using a Biotage Select purification system (Biotage Sfär C18, H₂O/MeOH, 60:40, to H₂O/MeOH, 20:80, over 32.8 column volumes, 6.0 mL/min). The purity (>96%) was determined by HPLC analysis (Eclipse XDB-C18, MeOH/H₂O, 77:23, 1.0 mL/min); R₂ (hexane/EtOAc, 60:40) = 0.14; [α]²⁰° +8.1 (c 0.5, MeOH); UV (MeOH) λmax (log ε) 263 (4.52), 273 (4.59), 284 (4.53) nm; ¹H NMR (400 MHz, CDCl₃) δ 6.56 (dd, J = 14.3, 11.3 Hz, 1H), 6.36 (dd, J = 10.8, 11.1 Hz, 1H), 6.25 (dd, J = 14.6, 10.6 Hz, 1H), 6.03 (apparent t, J = 11.1 Hz, 1H), 5.82 (dd, J = 15.0, 7.1 Hz, 1H), 5.55–5.25 (m, 5H), 4.01 (dd, J = 7.0, 4.9, 1.1 Hz, 1H), 3.65 (s, 3H), 3.54 (dt, J = 8.1, 4.9 Hz, 1H), 2.95 (t, J = 7.5, 2H), 2.48–2.25 (m, 3H), 2.22–1.97 (m, 5H), 1.61 (quint, J = 7.5 Hz, 2H), 1.47–1.23 (m, 4H), 0.97 (t, J = 7.5, 3.5 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 176.0, 134.4, 133.4, 133.7, 133.6, 131.4, 131.3, 129.1, 128.6, 126.3, 76.3, 76.0, 51.2, 34.8, 31.8, 30.9, 29.8, 28.0, 27.1, 25.9, 21.7, 14.6; HRESIMS m/z 299.2505 [M + Na]⁺ (calcd for C₉₂H₁₄₀O₄Na, 399.2506).

(7Z,10Z,12E,14E,19R)-16,17-Dihydroxydocosa-7,10,12,14,19-pentaenoic Acid (PD₂n-3_DPA, 2). Solid LiOH (6.7 mg, 0.28 mmol, 35 equiv) was added at 0 °C to a solution of the methyl ester 21 (3.0 mg, 8.0 µmol, 1.0 equiv) in THF/MeOH/H₂O (2:2:1, 1.1 mL). The mixture was stirred at 0 °C for 20 h. The solution was acidified with aqueous saturated NaHCO₃ (1.5 mL) before EtOAc (1.5 mL) was added. The layers were separated, and the water phase was extracted with EtOAc (2 × 2.0 mL). The combined organic layer was dried (Na₂SO₄) before being concentrated in vacuo. PD₂n-3_DPA (2) (2.6 mg, 7.2 µmol, 91%) was obtained after purification by column chromatography (4% MeOH in CH₂Cl₂) as a colorless oil: R₂ (hexane/EtOAc, 40:60) = 0.28; [α]²⁰° +22 (c 0.14, MeOH); UV (MeOH) λmax (log ε) 262 (4.52), 272 (4.59), 283 (4.53) nm; ¹H NMR (400 MHz, CDCl₃) δ 6.57 (dd, J = 14.3, 11.2 Hz, 1H), 6.36 (dd, J = 15.1, 10.7, 1.1 Hz, 1H), 6.26 (dd, J = 14.6, 10.7 Hz, 1H), 6.03 (apparent t, J = 11.1 Hz, 1H), 5.83 (dd, J = 15.0, 7.1 Hz, 1H), 5.50–5.32 (m, 3H), 4.01 (dd, J = 7.0, 4.9, 1.1 Hz, 1H), 3.55 (dd, J = 8.1, 4.9 Hz, 1H), 2.96 (t, J = 7.3 Hz, 2H), 2.39–2.26 (m, 3H), 2.31–2.03 (m, 5H), 1.62 (p, J = 7.3 Hz, 2H), 1.46–1.29 (m, 4H), 0.97 (t, J = 7.5 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 177.8, 134.4, 133.8, 133.7, 133.6, 131.5, 131.3, 129.6, 129.1, 128.5, 126.3, 76.3, 76.0, 35.1, 31.8, 30.4, 29.9, 28.0, 27.1, 26.1, 21.7, 14.6; HRESIMS m/z 385.2349 [M + Na]⁺ (calcd for C₉₂H₁₄₀O₄Na, 385.2349).
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