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Stereocontrolled total synthesis of neuroprotectin D1 / protectin D1 and its aspirin-triggered stereoisomer

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Abstract

Neuroprotectin D1 / protectin D1, a potent anti-inflammatory, proresolving, and neuroprotective lipid mediator derived biosynthetically from docosahexaenoic acid, was prepared in enantiomerically pure form via total organic synthesis. The synthetic strategy is highly stereocontrolled and convergent, featuring epoxide opening of glycidol starting materials for the introduction of the 10(R) and 17(S) hydroxyl groups. The desired alkene Z geometry was secured via the cis-reduction of alkyne precursors, while the conjugated E,E,Z triene was introduced at the end, in order to minimize Z/E isomerization. The same strategy, was also employed for the total synthesis of aspirin-triggered neuroprotectin D1 / protectin D1 having the 17(R)-stereochemistry. Synthetic compounds obtained with the reported method were matched with endogenously derived materials, and helped establish their complete stereochemistry.

Keywords

Neuroprotectin D1 / Protectin D1; Docosahexaenoic acid; Aspirin; Anti-inflammatory; Total synthesis

Recent investigations on the enzymatic oxygenation pathways of docosahexaenoic acid (DHA) led to the discovery of several novel lipid mediators, including the resolvins and protectins, which are potent endogenous anti-inflammatory and proresolving agents.¹ These findings provided the first supporting evidence at the molecular level for the long-recognized beneficial actions of DHA, a major omega-3 fatty acid, against several

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inflammatory² and neurodegenerative diseases.³ Since the isolation of such lipid mediators from biogenic sources typically generates very small quantities, it is often necessary to produce these molecules in larger scale in order to unambiguously establish their complete stereochemistry and to enable the detailed investigation of their biological actions and their potential use in drug discovery.

Herein, we detail the stereocontrolled total synthesis of a DHA-derived docosatriene^{4,5} (Fig. 1), that was initially named neuroprotectin D1 (NPD1)⁶ due to its potent actions in protecting the retina and the brain from oxidative stress. The same endogenously produced compound was also shown to have a broader range of activities in non-neuronal tissues and termed protectin D1 (PD1).⁷ The biosynthetic conversion of DHA to NPD1/PD1 (**1**) involves the action of 15-lipoxygenase (15-LO) to form an epoxide intermediate followed by enzymatic hydrolysis.⁸

A related enzymatic pathway that was recently elucidated,⁹ involves the initial oxygenation of DHA by cyclooxygenase-2 (COX-2) in the presence of aspirin (Fig. 1). Under these conditions, COX-2 functions as a lipoxygenase-like enzyme, catalyzing the stereospecific formation of hydroxylated products with a 17R, rather than a 17S stereochemistry. The resulting isomeric lipid mediator was termed aspirin-triggered NPD1/PD1 or AT-(NPD1/PD1) (**2**),⁹ and its synthesis is also reported herein.

In order to ensure the unambiguous total synthesis of NPD1/PD1 (**1**) in enantiomerically pure form, we earlier devised a convergent strategy^{8,10} by starting with protected glycidol and by generating all Z-alkenes via the selective reduction of the corresponding alkynes (Fig. 2). Thus, the synthesis of **1** involved selective reduction of diyne **3**, which is generated via the Sonogashira coupling of alkyne **4** and dienyliodide **5**. The chiral alcohol moieties of both intermediates **4** and **5** were produced via the initial epoxide opening of a glycidol derivative (**7** or **8**) with a metallated 1-alkyne (**6** or **7**). In addition to being highly convergent, this approach ensures a pre-defined R/S chirality depending on the glycidol enantiomer used, while all C=C bonds adopt the desired Z or E geometry and limit Z/E isomerization, particularly for the triene moiety.

The synthesis of the intermediate **4** is detailed in Fig. 3. Lithiation of 1-butyne followed by addition to the epoxide of the (S)-glycidol derivative **7** in the presence of boron trifluoride etherate, gave the resulting alcohol which was silylated to give the diprotected diol **10**. Selective removal of the primary silyl ether protective group of **10** with camphorsulfonic acid (CSA) to form **11** and subsequent Lindlar hydrogenation afforded alcohol **12** in high yield. Swern oxidation of **12** gave aldehyde **13** which was converted to alkyne **4**¹¹ via the Corey-Fuchs reaction.¹²

The synthesis of intermediate **5**, shown in Fig. 4, begun with a similar lithiation of silylated propargyl alcohol **9** followed by reaction with the (R)-glycidol derivative **8** to produce alcohol **14**. Protection of **14** as the *tert*-butyldiphenylsilyl ether (TBDPS) followed by treatment with CSA removed both primary *tert*-butyldimethylsilyl (TBS) groups to afford diol **15**. Selective bromination of the propargyl hydroxyl group with N-bromosuccinimide and triphenylphosphine, followed by silylation with TBS triflate and lutidine gave bromide **16**. Copper-mediated coupling of **14** with methyl pen-4-ynate **17**¹³ followed by removal of the primary TBS group gave alcohol **18**. Lindlar hydrogenation followed by Swern oxidation led to aldehyde **19**. Wittig homologation of **19** to the corresponding α,β -unsaturated aldehyde **20**, and Takai olefination^{14,15} gave the key E,E-dienyliodide intermediate **5**.¹⁶ Small amounts of the isomeric E,Z byproduct was removed with column chromatography, to afford **5** with greater isomeric purity.

For the combination of alkyne **4** and dienyl iodide **5** (Fig. 5), we relied on the palladium-catalyzed Sonogashira coupling reaction that proceeded in very high yield. Subsequent fluoride-mediated removal of the two silyl protective groups gave the alkynyl precursor of NPD1/PD1 methyl ester (**3**).¹⁷

The use of Lindlar hydrogenation for the conversion of the E,E-dienyne system of **3** to the corresponding E,E,Z triene is hampered by over-reduction and the formation of difficult to remove by-products. For this key step, we relied on the selective cis-reduction of the alkyne bond of **3** by using a known Zn(Cu/Ag) reagent^{18,19} freshly prepared from zinc dust, copper(II) acetate monohydrate and silver(I) nitrate. Unlike catalytic hydrogenation reactions, this alkyne reduction method is much more selective and it reduces alkynes in conjugated systems much faster than other alkynes or alkenes. Addition of excess of this heterogeneous reagent to a solution of **3** in aqueous methanol, followed by filtration and purification by HPLC, afforded NPD1/PD1 methyl ester (**21**)²⁰ in a stereochemically pure form. Hydroxide hydrolysis of **21** and HPLC purification gave NPD1/PD1 (**1**)²¹ as the sodium salt or as the free acid upon neutralization.

The aspirin-triggered isomer AT-(NPD1/PD1) (**2**) was synthesized similarly from its methyl ester (**24**), which was analogously prepared by reacting **5** with alkyne **22** instead of **4**. The 17R-stereochemistry of **22** was secured similarly to the synthesis of **4** (Fig. 3), by reacting alkyne **6** with the (R)-glycidol derivative **8** rather than **7**. Sonogashira coupling of **22** with iodide **5** followed by deprotection gave the diyne methyl ester precursor **23**,²² which was selectively reduced to the triene methyl ester **24**.²³ Hydrolysis of **24** gave AT-(NPD1/PD1) (**2**).²⁴

The methodology detailed herein was employed in the first total synthesis of NPD1/PD1 (**1**)^{8,10} and served as a means to unambiguously establish its complete stereochemical structure. The synthetic **1** was matched in all respects with material isolated from peripheral blood mononuclear cells,⁸ and was also used for the development of methods for lipidomic analysis via mass spectrometry.²⁵ Several enantiomerically pure stereoisomers of **1**, prepared by analogous synthetic routes, were utilized in detailed matching studies in comparison with biogenically-derived compounds. Based on these comparisons, we were able to assign for the first time the R/S and Z/E stereochemistry of this potent lipid mediator.⁸ Similarly, we recently employed this approach to firmly establish the stereochemistry of AT-(NPD1/PD1) (**2**).⁹

Synthetic protectins **1** and **2**, produced in larger quantities using the strategy detailed herein, were also employed in several collaborative studies aimed at exploring their biological actions and therapeutic potential against inflammation^{8,9} and neurodegenerative diseases affecting the retina^{26,27} or the brain, such as Alzheimer's disease.²⁸ These efforts provide further insights regarding the biological roles of these potent lipids, and may lead to a greater understanding of the important role of DHA and other omega-3 fatty acids in health and disease.

Acknowledgments

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11. Compound **4**: ^1H NMR(400MHz, CDCl_3): δ 7.72(m, 4H), 7.37(m, 6H), 5.46(m, 1H), 5.37(m, 1H), 4.31(td, $J=6.6\text{Hz}$ and 2.1Hz , 1H), 2.40(m, 2H), 2.33(d, $J=2.1\text{Hz}$, 1H), 1.28(m, 2H), 1.08(s, 9H), 0.88(td, $J=7.0\text{Hz}$ and 1.8Hz , 3). ^{13}C NMR (100MHz, CDCl_3): δ 136.0, 135.9, 134.6, 133.8, 133.6, 129.8, 128.6, 128.5, 127.6, 127.4, 84.8, 72.5, 63.6, 36.1, 26.9, 22.4, 14.1.
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16. Compound **5**: ^1H NMR(400MHz, CDCl_3): δ 7.67(m, 4H), 7.38(m, 6H), 6.93(dd, $J=15.1\text{Hz}$ and 11.2Hz , 1H), 6.18(d, $J=14.6\text{Hz}$, 1H), 5.90(dd, $J=14.6\text{Hz}$ and 10.8Hz , 1H), 5.67(dd, $J=15.1\text{Hz}$ and 6.4Hz , 1H), 5.32(m, 4H), 4.20(m, 1H), 2.62(m, 2H), 2.30(m, 6H), 1.09(s, 9H). ^{13}C NMR (100MHz, CDCl_3): δ 1743.4, 144.6, 136.5, 135.9, 135.8, 134.0, 133.7, 129.8, 129.7, 129.6, 129.5, 129.2, 127.7, 127.5, 127.4, 124.8, 73.0, 51.5, 35.6, 34.1, 27.0, 25.6, 19.3, 14.1.
17. Compound **3**: ^1H NMR (400MHz, CDCl_3): δ 6.57(dd, $J=15.6\text{Hz}$ and 10.8Hz , 1H), 6.29(dd, $J=15.8\text{Hz}$ and 11.4Hz , 1H), 5.83(dd, $J=15.4\text{Hz}$ and 5.8Hz , 1H), 5.7–5.3(m, 7H), 4.53(m, 1H), 4.24(m, 1H), 3.67(s, 3H), 2.84(m, 2H), 2.50(m, 2H), 2.38(m, 6H), 2.09(m, 2H), 0.98(t, $J=7.5\text{Hz}$, 3H). ^{13}C NMR (125MHz, CDCl_3): δ 170.65, 141.34, 138.11, 136.01, 131.49, 129.27, 128.94, 128.10, 124.63, 122.65, 110.79, 92.33, 83.96, 71.46, 62.54, 50.89, 35.59, 35.30, 33.92, 25.78, 22.82, 20.80, 14.22.
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20. NPD1/PD1 methyl ester (**21**): ^1H NMR (400MHz, MeOH-d_4): δ 6.51(dd, $J=14.0\text{Hz}$ and 11.5Hz , 1H), 6.26(m, 2H), 6.07(dd, $J=11.1\text{Hz}$ and 11.1Hz , 1H), 5.50–5.28(m, 7H), 4.90(s, 2H), 5.50–5.60(m, 2H), 4.55(m, 1H), 4.14(m, 1H), 3.65(s, 3H), 2.82(m, 2H), 2.40–2.13(m, 8H), 2.06(m, 2H), 2.07(m, 2H), 0.96(t, $J=7.5\text{Hz}$, 3H). ^{13}C NMR (125Hz, MeOH-d_4): δ 174.93, 137.59, 134.56, 134.47, 134.35, 131.01, 130.52, 130.17, 129.92, 128.57, 128.52, 126.14, 124.89, 72.60, 68.18, 36.00, 35.97, 34.45, 26.30, 23.43, 21.312, 14.20.
21. NPD1/PD1 (**1**): ^1H NMR (400MHz, MeOH-d_4): δ 6.52(dd, $J=14.0\text{Hz}$ and 11.5Hz , 1H), 6.26(m, 2H), 6.05(dd, $J=11.1\text{Hz}$ and 11.1Hz , 1H), 5.75(dd, $J=6.4$ and 14.4Hz , 1H), 5.50–5.30(m, 6H), 4.53(m, 1H), 4.04(m, 1H), 2.82(m, 2H), 2.35(m, 4H), 2.18(m, 4H), 2.05(m, 2H), 0.96(t, $J=7.5\text{Hz}$, 3H). ^{13}C NMR (400Hz, MeOH-d_4): δ 174.94, 137.58, 134.32, 134.48, 134.25, 130.71, 130.12, 129.92, 129.65, 127.92, 127.78, 124.84, 123.39, 71.80, 67.68, 35.35, 33.90, 31.54, 25.77, 22.80, 22.61, 14.10. MS (m/z): 137, 153, 163, 188, 206, 217, 245, 261, 279, 297, 315, 323, 341, 359.
22. Compound **23**: ^1H NMR (400MHz, CDCl_3): δ 6.57(dd, $J=15.6\text{Hz}$ and 10.8Hz , 1H), 6.30(J=15.8 and 11.4Hz , 1H), 5.84(dd, $J=15.4$ and 5.8Hz , 1H), 5.70–5.30(m, 7H), 4.53(m, 1H), 4.24(m, 1H), 3.67(s, 3H), 2.83(m, 2H), 2.09(m, 2H), 2.38 (m, 6H), 2.09(m, 2H), 0.98(t, $J=7.5\text{Hz}$, 3H). ^{13}C NMR

- (400Hz, CDCl₃): δ 170.65, 141.34, 138.11, 136.01, 131.49, 129.27, 128.94, 128.10, 124.63, 122.65, 110.79, 92.33, 83.96, 71.46, 62.54, 50.89, 35.59, 35.30, 33.92, 25.78, 22.82, 20.82, 14.22.
23. AT-(NPD1/PD1) methyl ester (**24**): ¹H NMR (400MHz, CDCl₃): δ 6.51(dd, J=14.0Hz and 11.4Hz, 1H), 6.26(m, 2H), 6.09(dd, J=11.0 and 11.0 Hz, 1H), 5.62–5.30(m, 7H), 4.60(m, 1H), 4.23(m, 1H), 3.67(s, 3H), 2.83(m, 2H), 2.44–2.22(m, 8H), 2.07(m, 2H), 0.96(t, J=7.5Hz, 3H). ¹³C NMR (400Hz, CDCl₃): δ 173.64, 136.48, 135.32, 133.78, 133.46, 131.21, 130.32, 130.03, 129.02, 128.02, 127.78, 124.85, 123.49, 71.81, 67.70, 35.37, 33.94, 31.58, 25.79, 22.82, 22.64, 14.10.
24. AT-(NPD1/PD1) (**2**): ¹H NMR (400MHz, MeOH-d₄): δ 6.52(dd, J=14.0Hz and 11.4Hz, 1H), 6.26(m, 2H), 6.07(dd, 10.8 and 11.6Hz), 5.75(dd, J=6.4 and 14.4 Hz, 1H), 5.50–5.29(m, 6H), 4.58(m, 1H), 4.12(m, 1H), 2.84(m, 2H), 2.35(m, 4H), 2.18(m, 4H), 2.06(m, 2H), 0.96(t, J=7.5Hz, 3H). ¹³C NMR (400Hz, MeOH-d₄): δ 174.94, 137.58, 134.32, 134.48, 134.25, 130.71, 130.12, 129.92, 129.65, 127.92, 127.78, 124.84, 123.39, 71.80, 67.68, 35.35, 33.90, 31.54, 25.77, 22.80, 22.61, 14.10. MS (*m/z*): 137, 153, 163, 188, 206, 217, 245, 261, 279, 297, 315, 323, 341, 359.
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Figure 1.
Biosynthesis of NPD1/PD1 and AT-(NPD1/PD1)

Figure 2.
Retrosynthetic analysis of NPD1/PD1.

Figure 3.

Synthesis of key intermediate **4**. (a) $n\text{BuLi}$, $\text{BF}_3 \cdot \text{OEt}_2$, -78°C , THF, 84%; (b) TBDPS-Cl, imidazole, DMAP, rt, CH_2Cl_2 , 89%; (c) CSA, rt, $\text{CH}_2\text{Cl}_2/\text{MeOH}$, 78%; (d) $\text{H}_2/\text{Lindlar cat.}$, quinoline, rt, EtOAc, 95%; (e) DMSO, $(\text{COCl})_2$, Et_3N , -78°C , CH_2Cl_2 , 98%; (f) PPh_3 , CBr_4 , 0°C , CH_2Cl_2 , 72%; (g) $n\text{BuLi}$, Et_2O , 83%.

Figure 4.

Synthesis of key intermediate **5**. (a) *n*-BuLi, $\text{BF}_3 \cdot \text{Et}_2\text{O}$, -78°C , THF, 77%; (b) TBDPS-Cl, imid., DMAP, rt, CH_2Cl_2 , 97%; (c) CSA, rt, $\text{CH}_2\text{Cl}_2/\text{MeOH}$, 81%; (d) NBS, PPh_3 , 0°C , CH_2Cl_2 , 80%; (e) TBSOTf, lutidine, 0°C , CH_2Cl_2 , 89%; (f) CuI, NaI, K_2CO_3 , rt, DMF, 75%; (g) CSA, rt, $\text{CH}_2\text{Cl}_2/\text{MeOH}$, 88%; (h) $\text{H}_2/\text{Lindlar cat.}$, quinoline, rt, EtOAc, 68%; (i) DMSO, $(\text{COCl})_2$, Et_3N , -78°C , CH_2Cl_2 , 98%; (j) $\text{Ph}_3\text{P}=\text{CHCHO}$, PhMe, reflux, 2h, 90%; (k) CHI_3 , CrCl_2 , 0°C , THF, 67%.

Figure 5.

Synthesis of NPD1/PD1 (**1**) and AT-(NPD1/PD1) (**2**). (a) cat. $Pd(PPh_3)_4$, CuI, rt, PhH, 96%; (b) TBAF, THF, rt, 2h, 65%; (c) Zn(Cu/Ag), MeOH/H₂O, 40 °C, 60%; (d) NaOH, MeOH/H₂O, rt, 95%. Insert shows the HPLC chromatogram of the co-elution of methyl esters **24** (retention time 11.3 min) and **21** (retention time 15.2 min), using a C18 reverse phase column, eluent of 28% water in methanol, and a UV detector at 270 nm.