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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 an 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. © 2012 Elsevier Ltd. All rights reserved.

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 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 DHAderived 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

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A related enzymatic pathway that was recently elucidated,⁹ involves the initial oxygenation of DHA by cyclooxygenase-2 (COX-2)



Figure 1. Biosynthesis of NPD1/PD1 and AT-(NPD1/PD1).





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Figure 3. Synthesis of the key intermediate **4**. (a) ^{*n*}BuLi, BF₃·OEt₂, -78 °C, THF, 84%; (b) TBDPS–Cl, imidazole, DMAP, rt, CH₂Cl₂, 89%; (c) CSA, rt, CH₂Cl₂/MeOH, 78%; (d) H₂/Lindlar cat., quinoline, rt, EtOAc, 95%; (e) DMSO, (COCl)₂ Et₃N, -78 °C, CH₂Cl₂, 98%; (f) PPh₃, CBr₄, 0 °C, CH₂Cl₂, 72%; (g) ^{*n*}BuLi, Et₂O, 83%.

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 17*R*, rather than a 17*S* 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 the selective reduction of dienyne **3**, which is generated via the Sonogashira coupling of alkyne **4** and dienyl iodide **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.



Figure 4. Synthesis of the key intermediate **5.** (a) *n*-BuLi, BF₃·Et₂O, -78 °C, THF, 77%; (b) TBDPS–Cl, imid., DMAP, rt, CH₂Cl₂, 97%; (c) CSA, rt, CH₂Cl₂/MeOH, 81%; (d) NBS, PPh₃, 0 °C, CH₂Cl₂, 80%; (e) TBSOTf, lutidine, 0 °C, CH₂Cl₂, 89%; (f) Cul, Nal, K₂CO₃, rt, DMF, 75%; (g) CSA, rt, CH₂Cl₂/MeOH, 88%; (h) H₂/Lindlar cat., quinoline, rt, EtOAc, 68%; (i) DMSO, (COCl₂, Et₃N, -78 °C, CH₂Cl₂, 98%; (j) Ph₃P=CHCHO, PhMe, reflux, 2 h, 90%; (k) CHI₃, CrCl₂, 0 °C, THF, 67%.

The synthesis of the intermediate **4** is detailed in Figure 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 deprotected 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 Figure 4, began 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 tertbutyldimethylsilyl (TBS) groups to afford diol 15. Selective bromination of the propargyl hydroxyl group with N-bromo-succinimide and triphenylphosphine, followed by silylation with TBS triflate and lutidine gave bromide **16**. Copper-mediated coupling of 14 with methyl pen-4-ynate 17¹³ followed by the 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*-dienyl iodide 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



Figure 5. Synthesis of NPD1/PD1 (**1**) and AT-(NPD1/PD1) (**2**). (a) Cat. Pd(PPh₃)₄, Cul, rt, PhH, 96%; (b) TBAF, THF, rt, 2 h, 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.

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 byproducts. 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 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 17*R*-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 dienyne 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 the Alzheimer's disease.²⁸ These efforts provide further insights into 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.

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N.A.P is an inventor on patents assigned to the University of Southern California and licensed to Resolvyx Pharmaceuticals. C.N.S. is an inventor on patents assigned to Brigham and Women's Hospital (BWH) and licensed to Resolvyx Pharmaceuticals. N.G.B. is an inventor on patents assigned to LSU-HSC. N.A.P. and C.N.S. are co-founders of Resolvyx Pharmaceuticals and own equity in the company. C.N.S.' interests were reviewed and are managed by BWH and Partners HealthCare in accordance with their conflict of interest policies.

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- Compound 4: ¹H NMR(400 MHz, CDCl₃): δ 7.72(m, 4H), 7.37(m, 6H), 5.46(m, 1H), 5.37(m, 1H), 4.31(td, J = 6.6 Hz and 2.1 Hz, 1H), 2.40(m, 2H), 2.33(d, J = 2.1 Hz, 1H), 1.28(m, 2H), 1.08(s, 9H), 0.88(td, J = 7.0 Hz and 1.8 Hz, 3). ¹³C NMR (100 MHz, CDCl₃): 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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 Compound 5: ¹H NMR(400 MHz, CDCl₃): δ 7.67(m, 4H), 7.38(m, 6H), 6.93(dd, *J* = 15.1 Hz and 11.2 Hz, 1H), 6.18(d, *J* = 14.6 Hz, 1H), 5.90(dd, *J* = 14.6 Hz and 10.8 Hz, 1H), 5.67(dd, *J* = 15.1 Hz and 6.4 Hz, 1H), 5.32(m, 4H), 4.20(m, 1H), 2.62(m, 2H), 2.30(m, 6H), 1.09(s, 9H). ¹³C NMR (100 MHz, CDCl₃): 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 (400 MHz, CDCl₃): δ 6.57(dd, J = 15.6 Hz and 10.8 Hz, 1H), 6.29(dd, J = 15.8 Hz and 11.4 Hz, 1H), 5.83(dd, J = 15.4 Hz and 5.8 Hz, 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 Hz, 3H). ¹³C NMR (125 MHz, 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, 20.80, 14.22.
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- NPD1/PD1 methyl ester (21): ¹H NMR (400 MHz, MeOH-d₄): δ 6.51(dd, J = 14.0 Hz and 11.5 Hz, 1H), 6.26(m, 2H), 6.07(dd, J = 11.1 Hz and 11.1 Hz, 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 Hz, 3H). ¹³C NMR (125 Hz, MeOH-d₄): 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.

- NPD1/PD1 (1): ¹H NMR (400 MHz, MeOH-d₄): δ 6.52(dd, *J* = 14.0 Hz and 11.5 Hz, 1H), 6.26(m, 2H), 6.05(dd, *J* = 11.1 Hz and 11.1 Hz, 1H), 5.75(dd, *J* = 6.4 and 14.4 Hz, 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 Hz, 3H). ¹³C NMR (400 Hz, 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.
- 22. Compound **23**: ¹H NMR (400 MHz, CDCl₃): δ 6.57(dd, J = 15.6 Hz and 10.8 Hz, 1H), 6.30(J = 15.8 and 11.4 Hz, 1H), 5.84(dd, J = 15.4 and 5.8 Hz, 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 Hz, 3H). ¹³C NMR (400 Hz, 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.
- AT-(NPD1/PD1) methyl ester (24): ¹H NMR (400 MHz, CDCl₃): δ 6.51(dd, J = 14.0 Hz and 11.4 Hz, 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 = 7.5 Hz, 3H). ¹³C NMR (400 Hz, 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 (400 MHz, MeOH- d_4): δ 6.52(dd, J = 14.0 Hz and 11.4 Hz, 1H), 6.26(m, 2H), 6.07(dd, 10.8 and 11.6 Hz), 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 = 7.5 Hz, 3H). ¹³C NMR (400 Hz, 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.
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