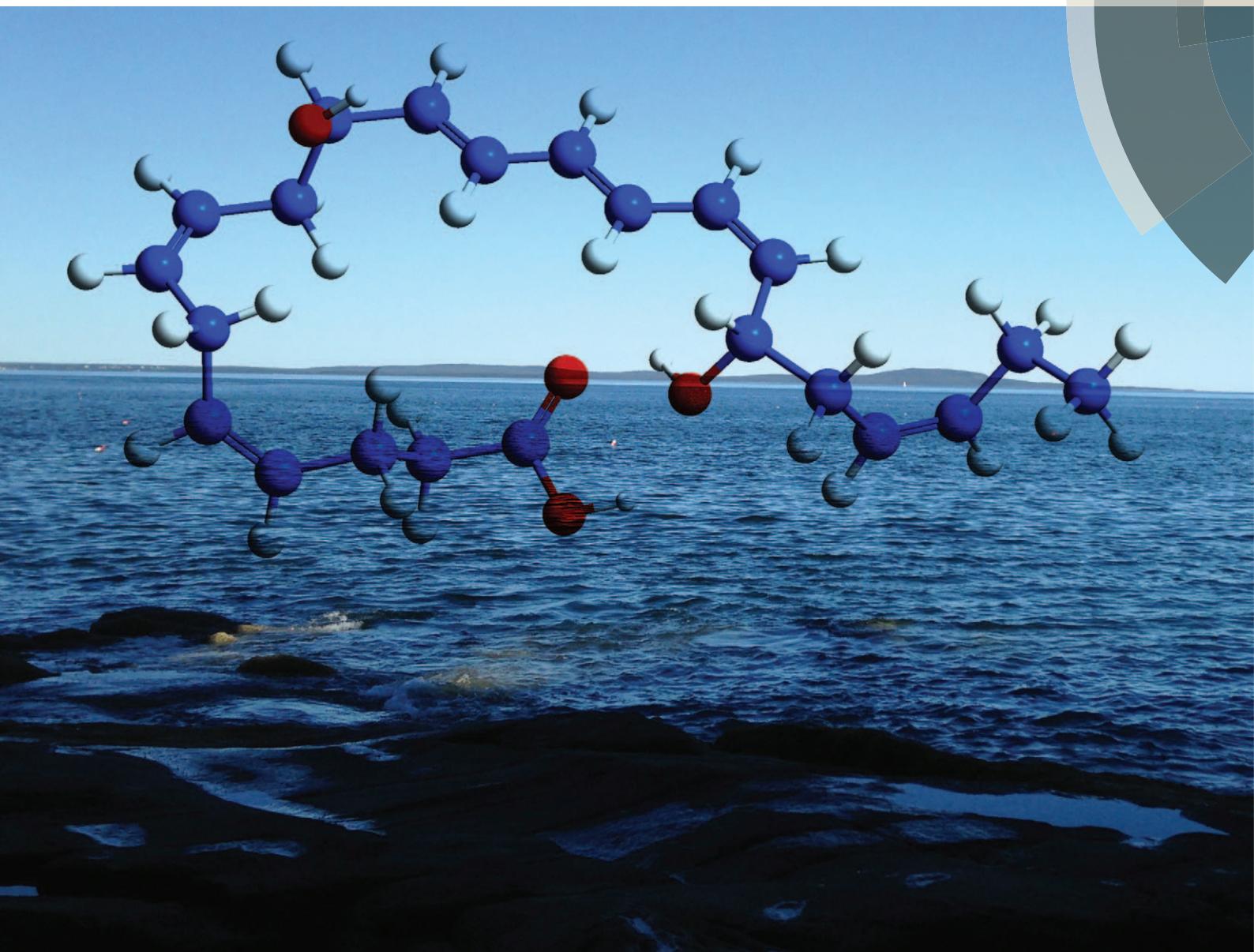


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PAPER

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Stereoselective synthesis of protectin D1: a potent anti-inflammatory and proresolving lipid mediator†

M. Aursnes,^a J. E. Tungen,^a A. Vik,^a J. Dalli^b and T. V. Hansen*^a

A convergent stereoselective synthesis of the potent anti-inflammatory, proresolving and neuroprotective lipid mediator protectin D1 (**2**) has been achieved in 15% yield over eight steps. The key features were a stereocontrolled Evans-aldol reaction with Nagao's chiral auxiliary and a highly selective Lindlar reduction of internal alkyne **23**, allowing the sensitive conjugated *E,E,Z*-triene to be introduced late in the preparation of **2**. The UV and LC/MS-MS data of synthetic protectin D1 (**2**) matched those obtained from endogenously produced material.

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Introduction

Polyunsaturated fatty acids (PUFAs), such as docosahexaenoic acid (**1**, DHA), play a major role in the physiology of living organisms.¹ Recent efforts by the Serhan research group have established that DHA (**1**) is a substrate for the biosynthesis of several potent anti-inflammatory proresolving mediators, such as protectin D1 (**2**),² maresin 1,³ resolvin D1 and resolvin D3.^{2a,4} All of these compounds have enabled new research areas related to many disease states associated with inflammation.⁵ It was reported that protectin D1 (**2**) is biosynthesized from DHA (**1**) via a lipoxygenase-mediated pathway that converts **1** by 15-lipoxygenase (15-LO) to the 17S-hydroperoxide intermediate (**3**), which is rapidly converted into the 16,17-epoxide (**4**), followed by enzymatic hydrolysis to the anti-inflammatory and proresolving oxygenated lipid **2** (Fig. 1).⁶

This compound has been reported to exhibit strong *in vivo* protective activity in several inflammatory⁶ as well as many other disease models.^{7–10} For example, the oxygenated polyunsaturated fatty acid **2** protects the retina and the brain from oxidative stress with very potent agonist activities.⁷ It is noteworthy that **2** was observed to be several orders of magnitude more potent *in vivo* than its precursor DHA.^{2c} Moreover, additional biological effects have recently been reported for this C22-oxygenated metabolite.¹¹ Hence, protectin D1 (**2**) is

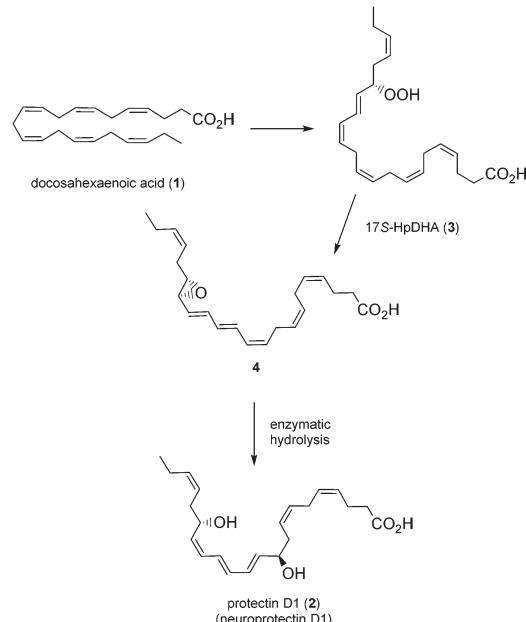


Fig. 1 Biosynthesis of protectin D1 (**2**).

very interesting as a lead compound for the development of potential new anti-inflammatory drugs.¹² The prefix *neuro* is added when this oxygenated PUFA is formed by neural tissues.^{2a} As of today, two syntheses of protectin D1 (**2**) have appeared.^{6,13} In connection with our interest in the synthesis of biologically active PUFA-derived natural products,¹⁴ as well as the many interesting biological activities of protectin D1 (**2**), we decided to prepare the DHA derived product **2**. A common structural feature for several of the lipid mediators isolated by the Serhan group^{2–4} is the chemically unstable *E,E,Z*-triene connected to either one or two secondary allylic alcohols. In the retrosynthetic analysis of **2**, Fig. 2, the aldehyde **6** is a key intermediate.

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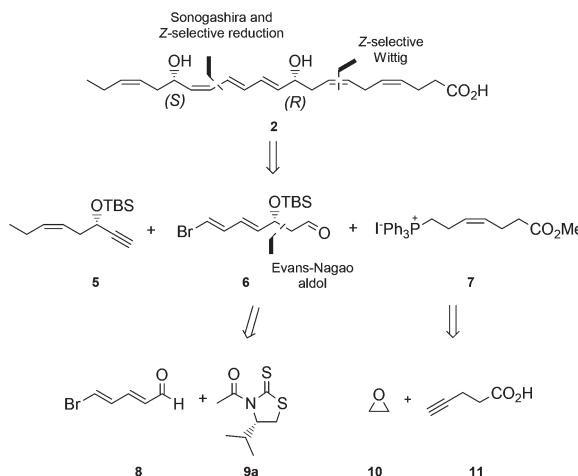
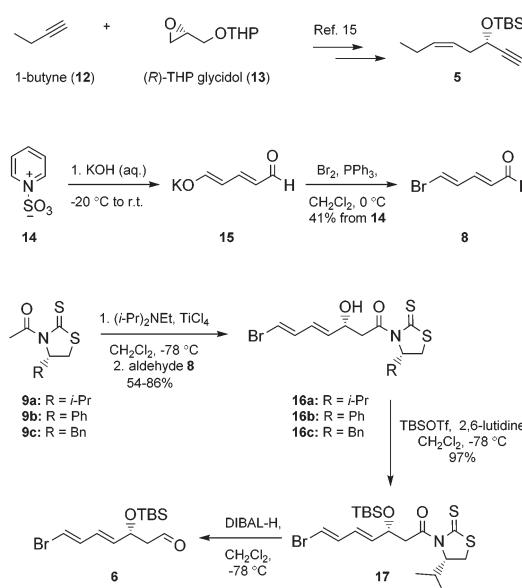


Fig. 2 Retrosynthetic analysis of protectin D1 (2).

Results and discussion

Our synthesis of **2** commenced with the preparation of **5**, essentially as previously reported,¹⁵ from 1-butyne (**12**) and THP-protected (*R*)-glycidol **13** (Scheme 1).

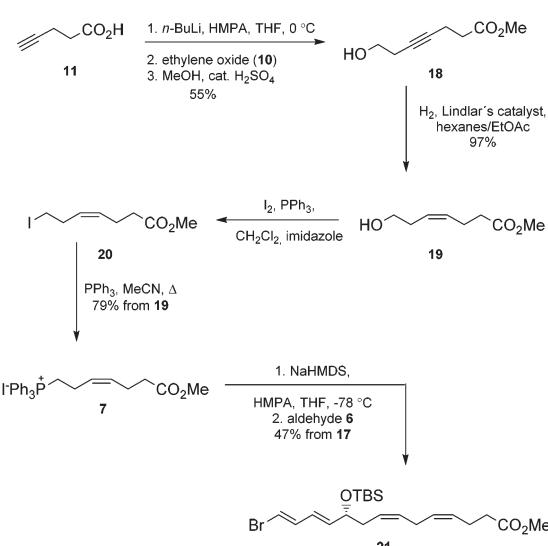
Aldehyde **8** was prepared by a slightly modified and improved literature protocol.¹⁶ Commercially available pyridinium-1-sulfonate (**14**) was treated with aqueous potassium hydroxide at $-20\text{ }^\circ\text{C}$ to yield glutaconaldehyde potassium salt **15** that was transformed further with the Br_2/PPh_3 complex to (*2E,4E*)-5-bromopenta-2,4-dienal (**8**) in 41% yield over the two steps. This sensitive aldehyde was then reacted with thiazolidinone **9a**, developed by Nagao and co-workers,¹⁷ in an Evans-aldol¹⁸ type reaction using conditions developed by Olivo and co-workers (TiCl_4 , $\text{Et}(\text{i-Pr})_2\text{N}$, CH_2Cl_2 , $-78\text{ }^\circ\text{C}$).¹⁹ This smoothly produced the intermediate **16a** in a 15.3:1 diastereomeric

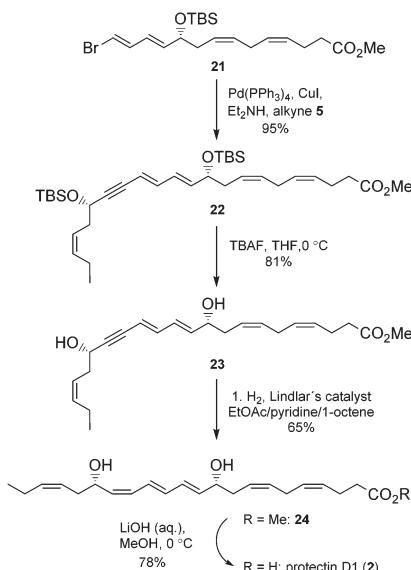
Scheme 1 Synthesis of alkyne **5** and aldehyde **6**.

ratio as determined by HPLC and ^1H NMR analyses. We also investigated reactions using thiazolidinones **9b** and **9c**, with the phenyl and the benzyl group, respectively, which afforded **16b** and **16c** with lower diastereoselectivity (4.5:1 and 9.8:1). Purification by chromatography yielded diastereomeric pure **16a** in 86% isolated yield. Protection of the alcohol functionality in **16a** to compound **17** was achieved using standard conditions.²⁰ Then DIBAL-H-reduction of **17** in CH_2Cl_2 at $-78\text{ }^\circ\text{C}$ afforded the sensitive aldehyde **6** (Scheme 1).

Next, the Wittig-salt **7** was synthesized. The dianion of 4-pentynoic acid (**11**) in HMPA,²¹ prepared by treatment with excess *n*-BuLi, was reacted with ethylene oxide (**10**). This afforded 7-hydroxy-hept-4-ynoic acid which was directly esterified to **18** (MeOH , catalytic H_2SO_4), see Scheme 2. Reduction of the internal alkyne in **18** using the Lindlar reaction gave (*Z*)-methyl 7-hydroxyhept-4-enoate (**19**) with high stereochemical purity as determined by ^1H NMR analyses. Then an Appel reaction²² provided the iodide **20** which was treated with PPh_3 in acetonitrile to provide the Wittig-salt **7** in a total yield of 42% from **11**. Conditions for the *Z*-stereoselective Wittig reaction between the key aldehyde **6** and the salt **7** were then investigated. Different bases, *i.e.* LiHMDS, KHMDS, NaHMDS, temperatures as well as altering the concentrations of **6** and **7**, with or without different amounts of HMPA in THF, all resulted in lower *Z*-selectivity. The best result was obtained when aldehyde **6** and the ylide of **7**, the latter obtained after treatment with NaHMDS in THF, were reacted at $-78\text{ }^\circ\text{C}$. This afforded the bromo-*E,E,Z,Z*-tetraene ester **21** (Scheme 2).

Chromatographic purification on silica gel yielded stereochemically pure product **21** (HPLC, $^1\text{H-NMR}$) in 47% yield over two steps. Then alkyne **5** was reacted with **21** in a Sonogashira reaction²³ at ambient temperature in the presence of $\text{Pd}(\text{PPh}_3)_4$ and CuI using diethyl amine as a solvent. This afforded the bis-hydroxyl-protected methyl ester **22** in 95% yield. Deprotection of the two TBS-groups in **22** was achieved with an excess of five equivalents of TBAF in THF at $0\text{ }^\circ\text{C}$ to

Scheme 2 Synthesis of ester **21**.



Scheme 3 Synthesis of protectin D1 (2).

afford 81% yield of the diol 23.²⁴ The internal conjugated alkyne in 23 was reduced to the methyl ester 24 in 65% yield after chromatographic purification on silica. A modified Lindlar hydrogenation reaction²⁵ produced triene 24 with high stereoselectivity, while the diimide reduction²⁶ or the standard Lindlar hydrogenation reaction²⁷ of 23 failed to give a high conversion to 24. The Boland reduction²⁸ gave in our hands a large amount of elimination of water from 23. Finally, lenient saponification of the methyl ester 24 at 0 °C with dilute aqueous LiOH in methanol followed by mild acidic work-up (aqueous NaH₂PO₄) afforded a 78% yield of protectin D1 (2) in the last step (Scheme 3).

The chemical purity of synthetic 2 and 24 was determined to be >95% and >98%, respectively, by HPLC analyses (see ESI†). The UV spectrum of synthetic protectin D1 (2) showed absorbance peaks ($\lambda_{\text{max}}^{\text{MeOH}}$) at 262, 271 and 282 nm, which is in excellent agreement with the literature.⁶ In order to obtain evidence that synthetic 2 and 24 matched that of authentic protectin D1 (2), protectin D1 (2) was obtained from endogenous murine self-resolving exudates.²⁹ Fig. 3 shows that the synthetic 2 was matched with endogenously produced 2.

In Fig. 3A authentic protectin D1 (2) obtained *in vivo* from exudates is displayed amongst its stereoisomers.³⁰ Fig. 3B shows the chromatographic behaviour of endogenously produced 2 ($T_{\text{R}} = 13.2$ min) and Fig. 3C demonstrates that synthetic 2 co-elutes with endogenous 2. In addition, the MS-MS spectra for both biosynthesized 2 and synthetic 2 displayed essentially identical MS-MS fragmentation spectra with the following fragments assigned: m/z 359 = M-H, m/z 341 = M-H-H₂O, m/z 323 = M-H-2H₂O, m/z 315 = M-H-CO₂, m/z 297 = M-H-H₂O-CO₂, m/z 279 = M-H-2H₂O-CO₂, m/z 243 = 261-H₂O, m/z 199 = 261-H₂O-CO₂, m/z 188 = 206-H₂O, m/z 135 = 153-H₂O, m/z 121 = 181-H₂O-CO₂, m/z 109 = 153-CO₂ (see ESI†). Similar results were also obtained when synthetic ester 24 was hydrolysed to the acid 2 and compared with authentic protectin

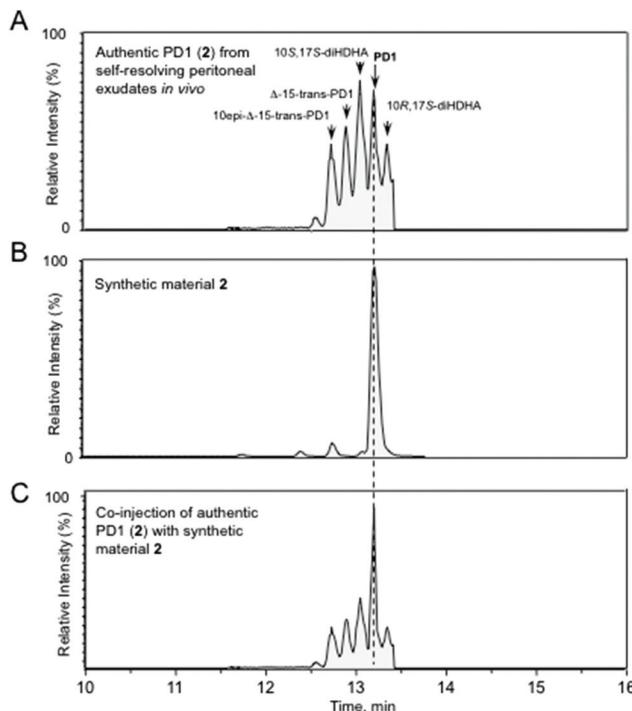


Fig. 3 HPLC chromatograms obtained from the matching experiments. Authentic protectin D1 (2) from self-resolving peritoneal inflammatory exudates matched synthetic material protectin D1 (2). Selected ion chromatograms (m/z 359–153) depicting (A) authentic protectin D1 (2), marked as PD1, obtained from mice injected with *Escherichia coli* (10^5 CFU) and exudates collected at 12 h; (B) synthetic protectin D1 (2) and (C) co-injection of protectin D1 (2) from self-resolving inflammatory exudates with synthetic material protectin D1 (2). Figures (A)–(C) are representative HPLC chromatograms ($n = 4$).

D1 (2). The chromatographic properties of synthetic 2 and the free acid of 24, the latter obtained by hydrolysis with aqueous LiOH in THF,⁶ were matched with data of endogenously formed protectin D1 (2). These results demonstrated that hydrolyzed 24 co-elutes with authentic 2. Furthermore, the MS-MS spectra for both the free acid obtained from 24 and biosynthesized 2 displayed essentially identical MS-MS fragmentation spectra (see ESI†). Our NMR spectral data of synthetic 2 were in accord with those published by Petasis, Serhan and co-workers,^{13b} but not with the spectra published by others.^{13a}

Conclusions

In summary, the potent endogenously produced lipid mediator protectin D1 (2) was prepared in eight steps and in 15% yield from the known aldehyde 8 in a convergent manner. Our synthesis of 2 compares well with those previously reported with respect to yields and simplicity, affording multi-mg quantities of this potent and biologically interesting natural product. The synthetic material displayed identical chromatographic properties with endogenously produced protectin D1 (2). Further *in vivo* biological studies are ongoing and will be reported elsewhere.



Experimental

(*R,4E,6E*)-7-Bromo-3-((*tert*-butyldimethylsilyl)oxy)hepta-4,6-dienal (6)

Aldehyde **6** was prepared by a DIBAL-H reduction of the protected thiazolidinethione **17** according to the procedure of Olivo *et al.*^{19b} All spectroscopic and physical data were in full agreement with those reported in the literature.^{19b} $[\alpha]_D^{20} = 31.5$ ($c = 0.2$, CHCl_3); ^1H NMR (300 MHz, CDCl_3) δ 9.75 (t, $J = 2.2$ Hz, 1H), 6.69 (dd, $J = 13.4$, 10.8 Hz, 1H), 6.33 (d, $J = 13.6$ Hz, 1H), 6.16 (ddd, $J = 15.2$, 10.6, 1.3 Hz, 1H), 5.75 (ddd, $J = 15.3$, 5.9, 0.8 Hz, 1H), 4.66 (dd, $J = 6.8$, 5.5 Hz, 1H), 2.75–2.41 (m, 2H), 0.87 (s, 9H), 0.06 (s, 3H), 0.04 (s, 3H); ^{13}C NMR (75 MHz, CDCl_3) δ 201.2, 136.6, 136.1, 127.6, 109.6, 68.5, 51.4, 25.9, 14.3, –4.2, –4.9.

(*R,4E,6E*)-7-Bromo-3-hydroxy-1-((*S*)-4-isopropyl-2-thioxothiazolidin-3-yl)hepta-4,6-dien-1-one (16a)

The (*R*)-aldol product **16a** was prepared in 86% yield from dienal **8** and the auxiliary **9a** according to the procedure of Olivo and coworkers.^{19a} The diastereomeric ratio (15.3 : 1) on the crude product was determined by HPLC analysis (Eclipse XDB-C18, $\text{MeOH}-\text{H}_2\text{O}$ 70 : 30, 1.0 mL min^{–1}, $t_{\text{r}}(\text{minor}) = 8.65$ min and $t_{\text{r}}(\text{major}) = 10.85$ min). All spectroscopic and physical data were in full agreement with those reported in the literature.^{19a} $[\alpha]_D^{20} = 271.3$ ($c = 0.13$, CHCl_3); ^1H NMR (300 MHz, CDCl_3) δ 6.72 (dd, $J = 13.5$, 10.8 Hz, 1H), 6.35 (d, $J = 13.6$ Hz, 1H), 6.26 (ddd, $J = 15.3$, 10.8, 1.5 Hz, 1H), 5.79 (dd, $J = 15.3$, 5.4 Hz, 1H), 5.16 (dd, $J = 7.8$, 6.4 Hz, 1H), 4.76–4.65 (m, 1H), 3.70 (dd, $J = 17.6$, 3.1 Hz, 1H), 3.53 (dd, $J = 11.5$, 7.9 Hz, 1H), 3.29 (dd, $J = 17.6$, 8.6 Hz, 1H), 3.04 (dd, $J = 11.6$, 1.1 Hz, 1H), 2.93 (d, $J = 4.5$ Hz, 1H), 2.36 (dq, $J = 13.6$, 6.8 Hz, 1H), 1.07 (d, $J = 6.7$ Hz, 3H), 0.99 (d, $J = 6.9$ Hz, 3H); ^{13}C NMR (101 MHz, CDCl_3) δ 203.1, 172.3, 136.7, 134.8, 128.0, 109.6, 71.5, 68.1, 45.1, 31.0, 30.8, 19.2, 18.0.

(*R,4E,6E*)-7-Bromo-3-((*tert*-butyldimethylsilyl)oxy)-1-((*S*)-4-isopropyl-2-thioxothiazolidin-3-yl)hepta-4,6-dien-1-one (17)

According to the procedure of Corey and coworkers,³¹ the alcohol **16a** was protected with a TBS-group. Yield: 4.2 g (97%); $[\alpha]_D^{20} = 263$ ($c = 0.5$, CHCl_3); ^1H NMR (300 MHz, CDCl_3) δ 6.69 (dd, $J = 13.4$, 10.7 Hz, 1H), 6.31 (d, $J = 13.5$ Hz, 1H), 6.15 (dd, $J = 15.5$, 11.1 Hz, 1H), 5.79 (dd, $J = 14.9$, 6.6 Hz, 1H), 5.04 (t, $J = 7.0$ Hz, 1H), 4.75 (q, $J = 6.4$ Hz, 1H), 3.64 (dd, $J = 16.6$, 7.8 Hz, 1H), 3.47 (dd, $J = 10.9$, 7.9 Hz, 1H), 3.21 (dd, $J = 16.4$, 4.6 Hz, 1H), 3.03 (d, $J = 11.6$ Hz, 1H), 2.48–2.26 (m, 1H), 1.06 (d, $J = 7.2$ Hz, 3H), 0.97 (d, $J = 7.1$ Hz, 3H), 0.86 (s, 9H), 0.05 (s, 3H), 0.03 (s, 3H); ^{13}C NMR (75 MHz, CDCl_3) δ 202.9, 170.9, 136.8, 127.4, 109.1, 71.8, 69.8, 46.2, 31.0, 30.9, 25.9 (3C), 19.3, 18.2, 17.9, –4.2, –4.8.

Methyl (*R,4Z,7Z,11E,13E*)-14-bromo-10-((*tert*-butyldimethylsilyl)oxy)tetradeca-4,7,11,13-tetraenoate (21)

To the Wittig salt **7** (581 mg, 1.04 mmol, 1.0 equiv.) in THF (9.5 mL) was added mol. sieves and HMPA (1.5 mL) before NaHMDS (0.6 M in toluene, 1.0 equiv.) was slowly added at

–78 °C and then stirred for 5 min at 0 °C. Aldehyde **6** (prepared from DIBAL-H reduction of **17** as described above) was added at –78 °C. The solution was allowed to slowly warm up to room temperature in the dry ice/acetone bath for 24 h before it was quenched with phosphate buffer (10 mL, pH = 7.2). Et_2O (15 mL) was added and the phases were separated. The aqueous phase was extracted with Et_2O (2 × 15 mL) and the combined organic layers were dried (Na_2SO_4), before it was concentrated *in vacuo*. The crude product was purified by column chromatography on silica (hexanes–EtOAc 95 : 5) to afford the title compound **21** as a yellow oil. Yield: 217 mg (47% for two steps starting from **17**); TLC (hexanes–EtOAc 95 : 5, CAM stain): $R_f = 0.29$; $[\alpha]_D^{20} = –9.4$ ($c = 0.1$, MeOH); ^1H NMR (400 MHz, CDCl_3) δ 6.68 (dd, $J = 13.4$, 10.9 Hz, 1H), 6.27 (d, $J = 13.5$ Hz, 1H), 6.09 (dd, $J = 15.2$, 10.8 Hz, 1H), 5.72 (dd, $J = 15.2$, 5.8 Hz, 1H), 5.48–5.32 (m, 4H), 4.16 (q, $J = 6.0$ Hz, 1H), 3.67 (s, 3H), 2.84–2.73 (m, 2H), 2.38–2.35 (m, 4H), 2.35–2.21 (m, 2H), 0.89 (s, 9H), 0.05 (s, 3H), 0.02 (s, 3H); ^{13}C NMR (101 MHz, CDCl_3) δ 173.6, 138.0, 137.1, 129.9, 129.4, 128.0, 126.7, 125.6, 108.3, 72.6, 51.7, 36.3, 34.1, 26.0 (3C), 25.9, 23.0, 18.4, –4.4, –4.6. HRMS (TOF ES⁺): Exact mass calculated for $\text{C}_{21}\text{H}_{35}\text{O}_3\text{Si}^{79}\text{BrNa} [M + \text{Na}]^+$: 465.1436, found 465.1431.

Methyl (4*Z*,7*Z*,10*R*,11*E*,13*E*,17*S*,19*Z*)-10,17-bis((*tert*-butyldimethylsilyl)oxy)docosa-4,7,11,13,19-pentaen-15-ynoate (22)

To a solution of vinyl bromide **21** (218 mg, 0.49 mmol, 1.0 equivalent) in Et_2NH (1.2 mL) and benzene (0.4 mL), $\text{Pd}(\text{PPh}_3)_4$ (17 mg, 0.02 mmol, 3 mol%) was added and the reaction was stirred for 45 min in the dark. CuI (5 mg, 0.03 mmol, 5 mol%) in a minimum amount of Et_2NH was added followed by dropwise addition of alkyne **5** (117 mg, 0.49 mmol, 1.0 equiv.) in Et_3N (1.0 mL). After 20 h of stirring at ambient temperature, the reaction was quenched by the addition of saturated NH_4Cl (15 mL). Et_2O (15 mL) was added and the phases were separated. The aqueous phase was extracted with Et_2O (2 × 15 mL) and the combined organic layers were dried (Na_2SO_4), before being concentrated *in vacuo*. The crude product was purified by column chromatography on silica (hexanes–EtOAc 95 : 5) to afford the title compound **22** as a pale yellow oil. Yield: 278 mg (95%); TLC (hexanes–EtOAc 9 : 1, CAM stain): $R_f = 0.44$; $[\alpha]_D^{20} = –15.5$ ($c = 0.20$, MeOH); ^1H NMR (400 MHz, CDCl_3) δ 6.51 (dd, $J = 15.6$, 10.9 Hz, 1H), 6.19 (dd, $J = 15.2$, 10.8 Hz, 1H), 5.76 (dd, $J = 15.2$, 6.0 Hz, 1H), 5.58 (dd, $J = 15.3$, 1.2 Hz, 1H), 5.55–5.47 (m, 1H), 5.45–5.33 (m, 5H), 4.47 (td, $J = 6.5$, 1.6 Hz, 1H), 4.19 (q, $J = 6.3$ Hz, 1H), 3.67 (s, 3H), 2.82–2.74 (m, 2H), 2.43 (t, $J = 7.2$ Hz, 2H), 2.40–2.33 (m, 4H), 2.33–2.20 (m, 2H), 2.07 (p, $J = 7.4$ Hz, 2H), 0.97 (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, $J = 8.7$ Hz, 6H); ^{13}C NMR (101 MHz, CDCl_3) δ 173.7, 141.2, 139.2, 134.4, 129.8, 129.5, 128.7, 128.0, 125.8, 124.1, 110.7, 93.5, 83.5, 72.8, 63.7, 51.7, 36.8, 36.4, 34.2, 26.0 (3C), 25.9, 23.0, 20.9, 18.5, 18.4, 14.4, –4.3 (2C), –4.6, –4.8. HRMS (TOF ES⁺): Exact mass calculated for $\text{C}_{35}\text{H}_{60}\text{O}_4\text{Si}_2\text{Na} [M + \text{Na}]^+$: 623.3927, found 623.3923.



Methyl (4Z,7Z,10R,11E,13E,17S,19Z)-10,17-dihydroxydocosa-4,7,11,13,19-pentaen-15-ynoate (23)

TBAF (587 mg, 2.25 mmol, 5.0 equiv., 1.0 M in THF) was added to a solution of TBS-protected alcohol **22** (270 mg, 0.45 mmol, 1.0 equiv.) in THF (6.0 mL) at 0 °C. The reaction was stirred for 20 h before it was quenched with phosphate buffer (pH = 7.2, 3.5 mL). Brine (30 mL) and EtOAc (30 mL) were added and the phases were separated. The water phase was extracted with EtOAc (2 × 30 mL) and the combined organic layer was dried (Na_2SO_4) before being concentrated *in vacuo*. The crude product was purified by column chromatography on silica (hexanes-EtOAc 7:3) to afford the title compound **23** as a pale yellow oil. Yield: 135 mg (81%); TLC (hexanes-EtOAc 7:3, CAM stain): R_f = 0.19; $[\alpha]_D^{20} = -9.2$ ($c = 0.3$, MeOH); ^1H NMR (400 MHz, MeOD- d_4) δ 6.54 (dd, $J = 15.6, 10.8$ Hz, 1H), 6.29 (dd, $J = 15.2, 10.8$ Hz, 1H), 5.82 (dd, $J = 15.2, 6.2$ Hz, 1H), 5.66 (dd, $J = 15.1, 1.8$ Hz, 1H), 5.57–5.32 (m, 6H), 4.41 (t, $J = 6.7$ Hz, 1H), 4.14 (q, $J = 6.5$ Hz, 1H), 3.66 (s, 3H), 2.87–2.74 (m, 2H), 2.46–2.28 (m, 6H), 2.10 (p, $J = 7.4$ Hz, 2H), 0.98 (t, $J = 7.5$ Hz, 3H); ^{13}C NMR (101 MHz, MeOD- d_4) δ 175.3, 142.5, 139.8, 135.3, 131.0, 130.3, 130.3, 129.0, 126.4, 124.7, 111.8, 93.9, 84.3, 72.7, 63.3, 52.1, 36.9, 36.2, 34.8, 26.7, 23.8, 21.7, 14.6. HRMS (TOF ES $^+$): Exact mass calculated for $\text{C}_{23}\text{H}_{32}\text{O}_4\text{Na}$ [$M + \text{Na}^+$]: 395.2198, found 395.2206.

Methyl (4Z,7Z,10R,11E,13E,15Z,17S,19Z)-10,17-dihydroxy-dcosa-4,7,11,13,15,19-hexaenoate (24)

To a solution of alkyne **23** (30 mg, 0.082 mmol) in EtOAc-pyridine-1-octene (0.83 mL, 10:1:1) under argon, Lindlar's catalyst (10 mg) was added and the flask was evacuated and filled with argon. The reaction was stirred for 3.5 h at ambient temperature under a balloon of hydrogen gas until completion. The reaction mixture was loaded directly onto a silica gel column and purified by chromatography (hexanes-EtOAc 8:2) to afford the title compound **24** as a pale oil. The chemical purity (>98%) was determined by HPLC analysis (Eclipse XDB-C18, MeOH-H₂O 75:25, 1.0 mL min $^{-1}$): t_r (minor) = 12.62 min, and t_r (major) = 9.07 min. Yield: 19.5 mg (65%); TLC (hexanes-EtOAc 6:4, CAM stain): R_f = 0.19; $[\alpha]_D^{20} = -22.2$ ($c = 0.4$, MeOH); UV (MeOH) λ_{max} 262, 271, 282 nm. ^1H NMR (400 MHz, MeOD- d_4) δ 6.52 (dd, $J = 14.0, 10.7$ Hz, 1H), 6.33–6.18 (m, 2H), 6.07 (t, $J = 11.1$ Hz, 1H), 5.76 (dd, $J = 14.5, 6.5$ Hz, 1H), 5.49–5.32 (m, 7H), 4.56 (dt, $J = 8.9, 6.7$ Hz, 1H), 4.14 (q, $J = 6.5$ Hz, 1H), 3.65 (s, 3H), 2.87–2.78 (m, 2H), 2.40–2.29 (m, 7H), 2.25–2.16 (m, 1H), 2.07 (p, $J = 7.4$ Hz, 2H), 0.97 (t, $J = 7.5$ Hz, 3H); ^{13}C NMR (101 MHz, MeOD- d_4) δ 175.3, 138.0, 134.9, 134.9, 134.7, 131.4, 130.9, 130.5, 130.3, 128.9, 128.9, 126.5, 125.3, 73.0, 68.6, 52.1, 36.4, 36.4, 34.8, 26.7, 23.8, 21.7, 14.6. HRMS (TOF ES $^+$): Exact mass calculated for $\text{C}_{23}\text{H}_{34}\text{O}_4\text{Na}$ [$M + \text{Na}^+$]: 397.2354, found 397.2365. All spectroscopic and physical data were in agreement with those reported in the literature.^{13b}

Synthesis of protectin D1 (2)

Methyl ester **24** (18 mg, 0.032 mmol) was dissolved in methanol-water 1:1 (30 mL) and cooled to 0 °C. LiOH (1.0 M,

1.9 mL) was added dropwise. The reaction mixture was stirred at the above-mentioned temperature for 48 h, after which a saturated solution of NaH_2PO_4 (4.0 mL) was added. Next, NaCl (10.0 g) was added followed by EtOAc (50 mL). The organic phase was decanted, dried (Na_2SO_4), and concentrated *in vacuo* affording the title compound **2** (14 mg, 78%) as a colourless oil. The chemical purity (>95%) was determined by HPLC analysis (Eclipse XDB-C18, MeOH-3.3 mM HCOOH in H₂O, 7:3, 1.0 mL min $^{-1}$): t_r (minor) = 9.97 min and t_r (major) = 10.68 min; TLC (hexanes-EtOAc 6:4, CAM stain): R_f = 0.03; $[\alpha]_D^{20} = -24.0$ ($c = 0.3$, MeOH); UV (MeOH) λ_{max} 262, 271, 282 nm. IR (neat) ν = 3316, 3012, 2961, 2930, 1713, 1557 cm $^{-1}$; ^1H NMR (300 MHz, MeOH- d_4) δ 6.52 (dd, $J = 14.1, 11.3$ Hz, 1H), 6.35–6.19 (m, 2H), 6.08 (dd, $J = 11.7, 10.5$ Hz, 1H), 5.76 (dd, $J = 14.4, 6.5$ Hz, 1H), 5.52–5.31 (m, 7H), 4.56 (dt, $J = 9.4, 6.8$ Hz, 1H), 4.21–4.08 (m, 1H), 2.88–2.78 (m, 2H), 2.42–2.15 (m, 8H), 2.12–2.00 (m, 2H), 0.97 (t, $J = 7.5$ Hz, 3H); ^{13}C NMR (126 MHz, CDCl₃) δ 177.4, 137.9, 134.9, 134.8, 134.7, 131.4, 131.0, 130.6, 130.0, 129.3, 128.9, 126.5, 125.3, 73.0, 68.6, 36.4, 36.3, 35.3, 26.7, 24.0, 21.7, 14.6. HRMS (TOF ES $^-$): Exact mass calculated for $\text{C}_{22}\text{H}_{31}\text{O}_4$ [$M - \text{H}^-$]: 359.2222, found 359.2213. All spectroscopic and physical data were in agreement with those reported in the literature.^{13b}

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