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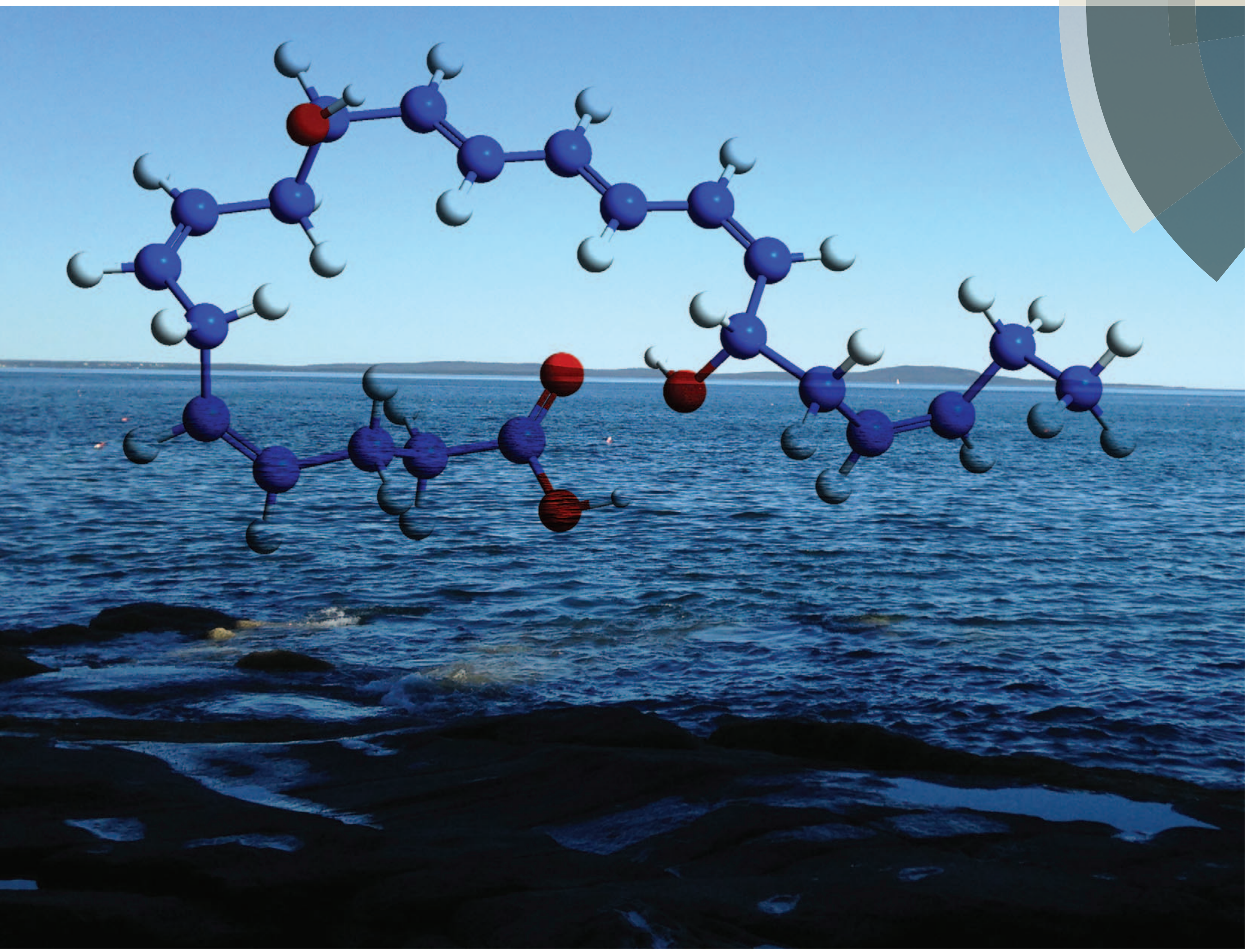

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PAPER

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

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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.

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 17*S*-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 inflammatory[6] 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 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.

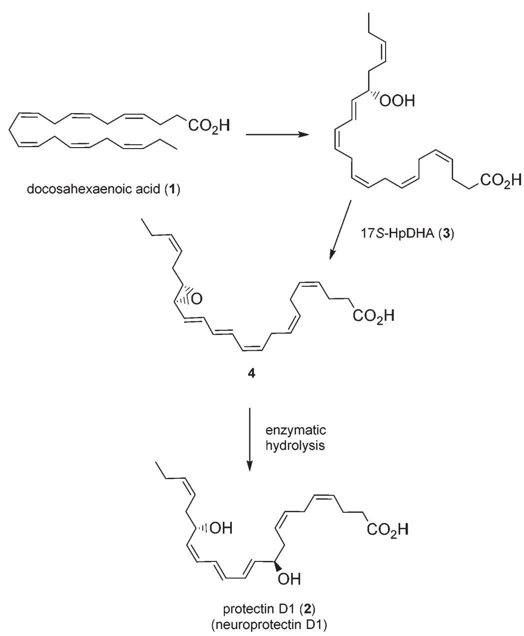

Fig. 1 Biosynthesis of protectin D1 (**2**).

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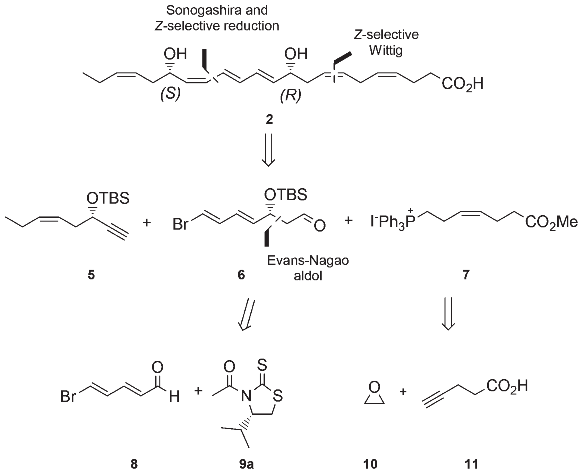


Fig. 2 Retrosynthetic analysis of protectin D1 (**2**).

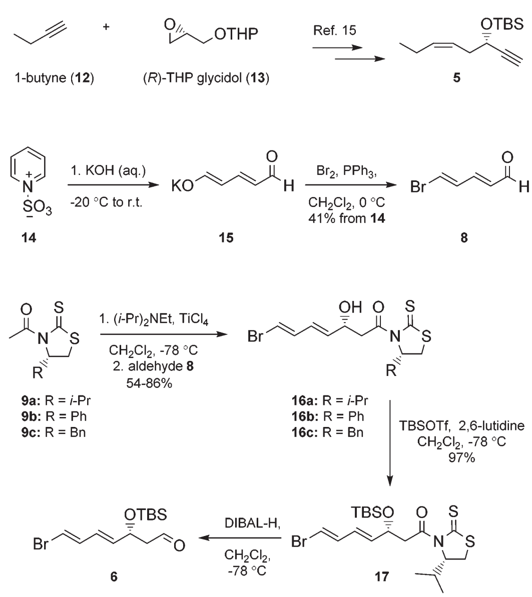
Results and discussion

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).

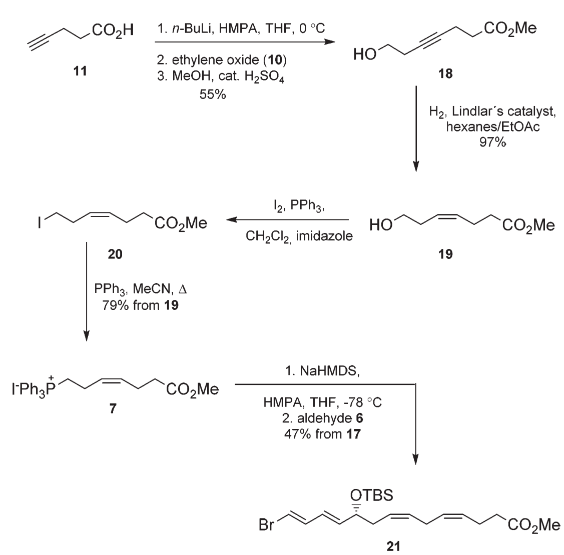
Aldehyde **8** was prepared by a slightly modified and improved literature protocol.[16] Commercially available pyridinium-1-sulfonate (**14**) was treated with aqueous potassium hydroxide at −20 °C to yield glutaconaldehyde potassium salt **15** that was transformed further with the Br_2/PPh_3 complex to (2*E*,4*E*)-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-aldol[18] type reaction using conditions developed by Olivo and co-workers ($TiCl_4$, Et(i-Pr)$_2$N, CH_2Cl_2, −78 °C).[19] This smoothly produced the intermediate **16a** in a 15.3 : 1 diastereomeric ratio as determined by HPLC and ^{1}H 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 °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 ^{1}H NMR analyses. Then an Appel reaction[22] 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 °C. This afforded the bromo-*E*,*E*,*Z*,*Z*-tetraene ester **21** (Scheme 2).

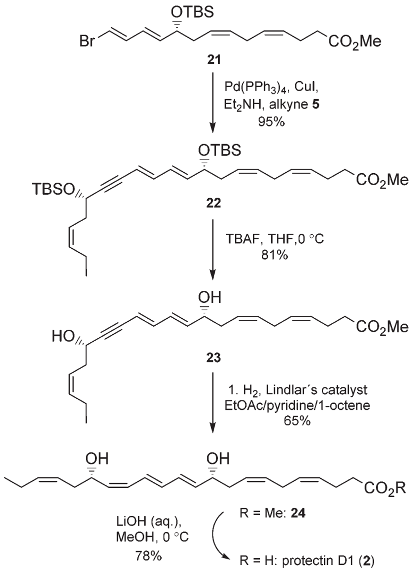
Chromatographic purification on silica gel yielded stereochemically pure product **21** (HPLC, ^{1}H-NMR) in 47% yield over two steps. Then alkyne **5** was reacted with **21** in a Sonogashira reaction[23] at ambient temperature in the presence of Pd(PPh_3)$_4$ and CuI using diethyl amine as a solvent. 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 °C to



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



Scheme 2 Synthesis of ester **21**.



Scheme 3 Synthesis of protectin D1 (**2**).

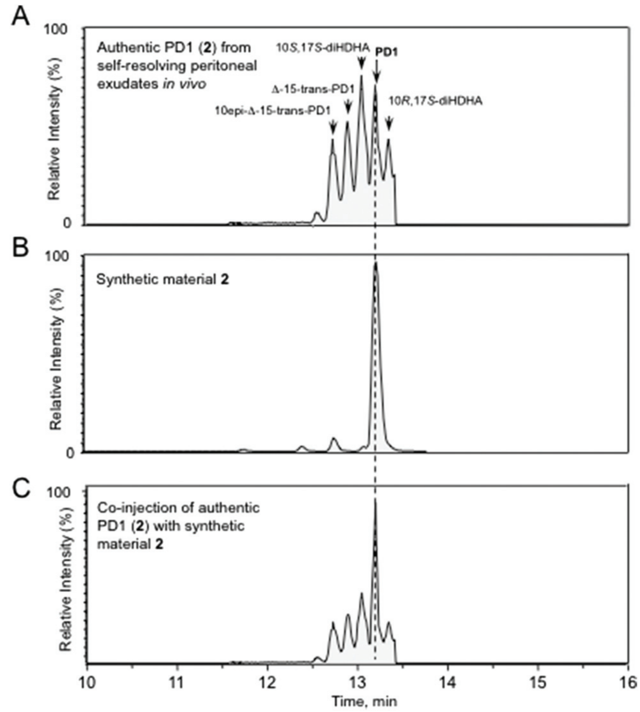


Fig. 3 HPLC chromatograms obtained from the matching experiments. Authentic protectin D1 (**2**) from self-resolving peritoneal inflammatory exudates matched synthetic material protectin D1 (**2**). Selected ion chromatograms (*m*/*z* 359–153) depicting (A) authentic protectin D1 (**2**), marked as PD1,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

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 reaction[25] produced triene **24** with high stereoselectivity, while the diimide reduction[26] or the standard Lindlar hydrogenation reaction[27] of **23** failed to give a high conversion to **24**. The Boland reduction[28] 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 NaH_2PO_4) 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 (λ_{max}^{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_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-H_2O, *m*/*z* 323 = M-H-2H_2O, *m*/*z* 315 = M-H-CO_2, *m*/*z* 297 = M-H-H_2O-CO_2, *m*/*z* 279 = M-H-2H_2O-CO_2, *m*/*z* 243 = 261-H_2O, *m*/*z* 199 = 261-H_2O-CO_2, *m*/*z* 188 = 206-H_2O, *m*/*z* 135 = 153-H_2O, *m*/*z* 121 = 181-H_2O-CO_2, *m*/*z* 109 = 153-CO_2 (see ESI†). Similar results were also obtained when synthetic ester **24** was hydrolysed to the acid **2** and compared with authentic protectin 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*,4*E*,6*E*)-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*,4*E*,6*E*)-7-Bromo-3-hydroxy-1-((*S*)-4-isopropyl-2-thioxothia-zolidin-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, MeOH–H_2O 70 : 30, 1.0 mL min^{-1}, t_r(minor) = 8.65 min and t_r(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*,4*E*,6*E*)-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*,4*Z*,7*Z*,11*E*,13*E*)-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 $C_{21}H_{35}O_3Si^{79}BrNa$ $[M + Na]^+$: 465.1436, found 465.1431.

Methyl (4*Z*,7*Z*,10*R*,11*E*,13*E*,17*S*,19*Z*)-10,17-bis((*tert*-butyl-di-methylsilyl)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), $Pd(PPh_3)_4$ (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), 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 $C_{35}H_{60}O_4Si_2Na$ $[M + Na]^+$: 623.3927, found 623.3923.

Methyl (4*Z*,7*Z*,10*R*,11*E*,13*E*,17*S*,19*Z*)-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); ^{1}H 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 $C_{23}H_{32}O_4Na$ $[M + Na]^+$: 395.2198, found 395.2206.

Methyl (4*Z*,7*Z*,10R,11*E*,13*E*,15*Z*,17*S*,19*Z*)-10,17-dihydroxy-do-cosa-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–H_2O 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. ^{1}H 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 $C_{23}H_{34}O_4Na$ $[M + Na]^+$: 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 H_2O, 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}; ^{1}H 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, $CDCl_3$) 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 $C_{22}H_{31}O_4$ $[M - H]^-$: 359.2222, found 359.2213. All spectroscopic and physical data were in agreement with those reported in the literature.[13b]

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