Volume 12 | Number 3 | 21 January 2014 | Pages 385–542

Organic & Biomolecular Chemistry

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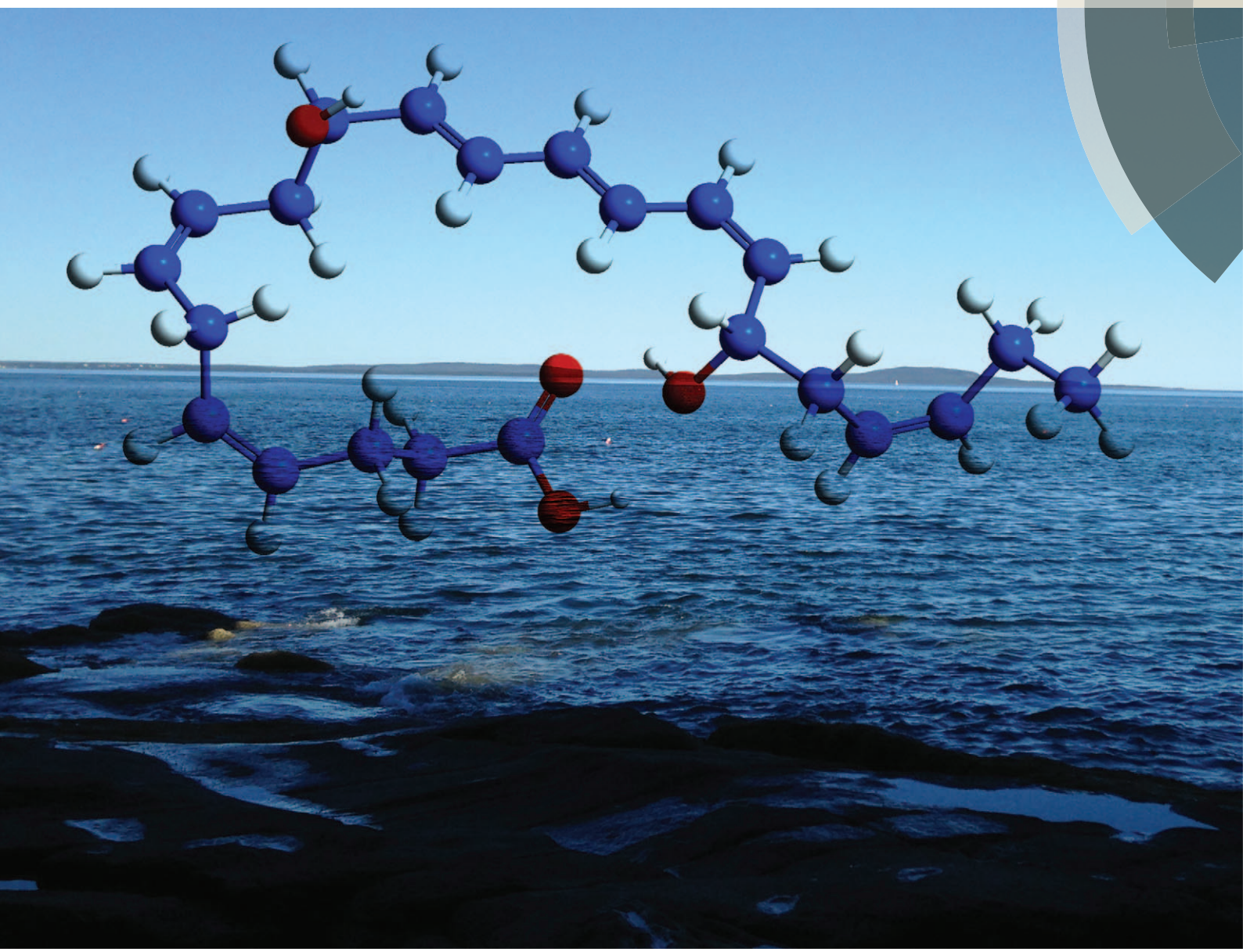

ISSN 1477-0520

ROYAL SOCIETY OF CHEMISTRY

PAPER
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Cite this: *Org. Biomol. Chem.*, 2014, **12**, 432

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

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Received 19th September 2013,
Accepted 31st October 2013

DOI: 10.1039/c3ob41902a

www.rsc.org/obc

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.

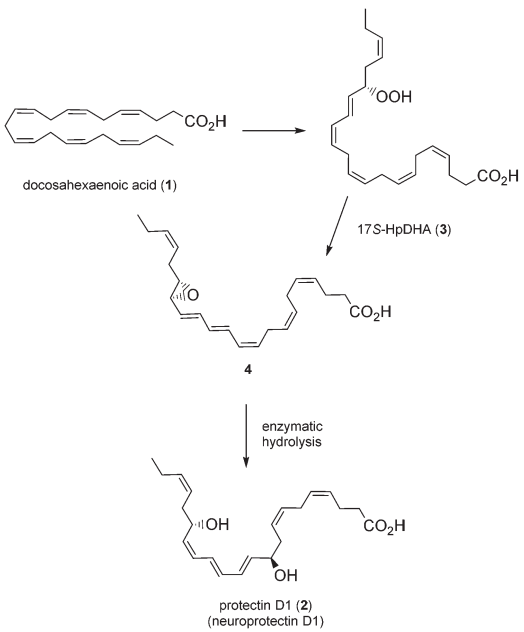

Fig. 1 Biosynthesis of protectin D1 (**2**).

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† Electronic supplementary information (ESI) available: Additional experimental procedures and characterization data, ^{1}H-, ^{13}C-NMR, HRMS, LC-MS/MS and UV/VIS spectra as well as chromatograms of HPLC analyses. See DOI: 10.1039/c3ob41902a

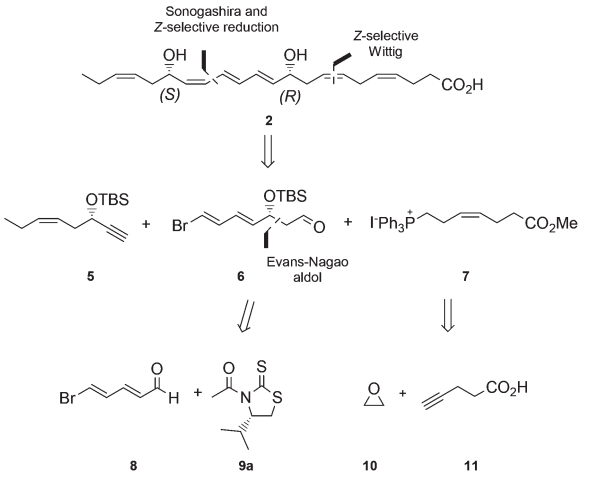


Fig. 2 Retrosynthetic analysis of protectin D1 (**2**).

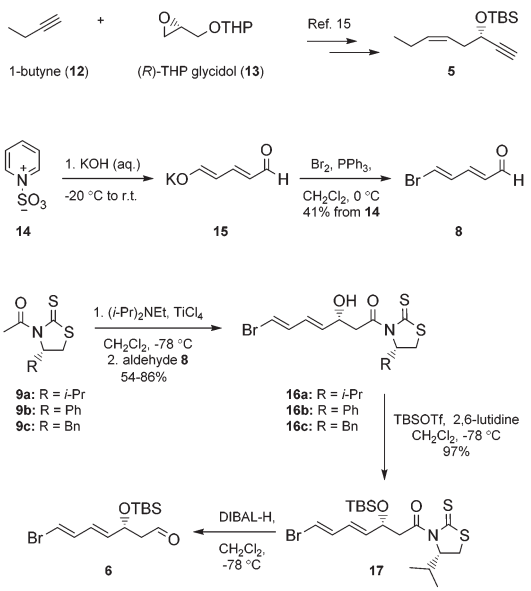
Results and discussion

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).

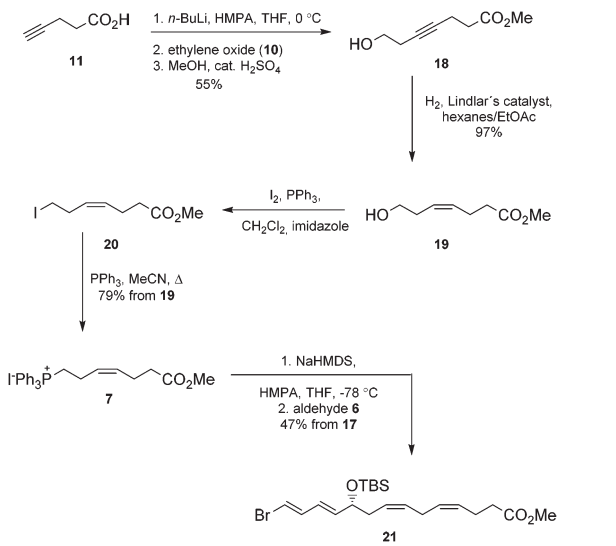
Aldehyde **8** was prepared by a slightly modified and improved literature protocol.[16] Commercially available pyridinium-1-sulfonate (**14**) was treated with aqueous potassium hydroxide at −20 °C to yield glutaconaldehyde potassium salt **15** that was transformed further with the Br_2/PPh_3 complex 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 ($TiCl_4$, $Et(i\text{-}Pr)_2N$, CH_2Cl_2, −78 °C).[19] This smoothly produced the intermediate **16a** in a 15.3 : 1 diastereomeric 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 °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 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).

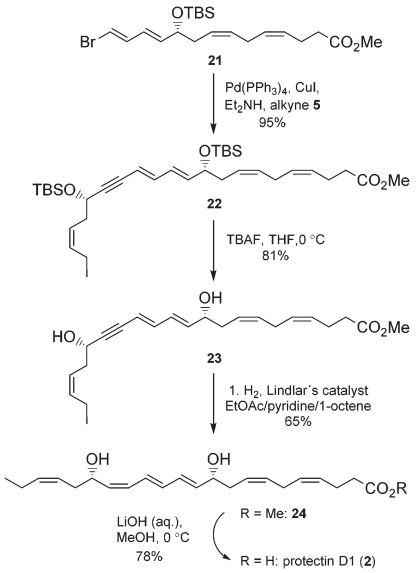
Chromatographic purification on silica gel yielded stereochemically pure product **21** (HPLC, ^1H-NMR) in 47% yield over two steps. Then alkyne **5** was reacted with **21** in a Sonogashira reaction[23] at ambient temperature in the presence of $Pd(PPh_3)_4$ and CuI using diethyl amine as a solvent.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

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

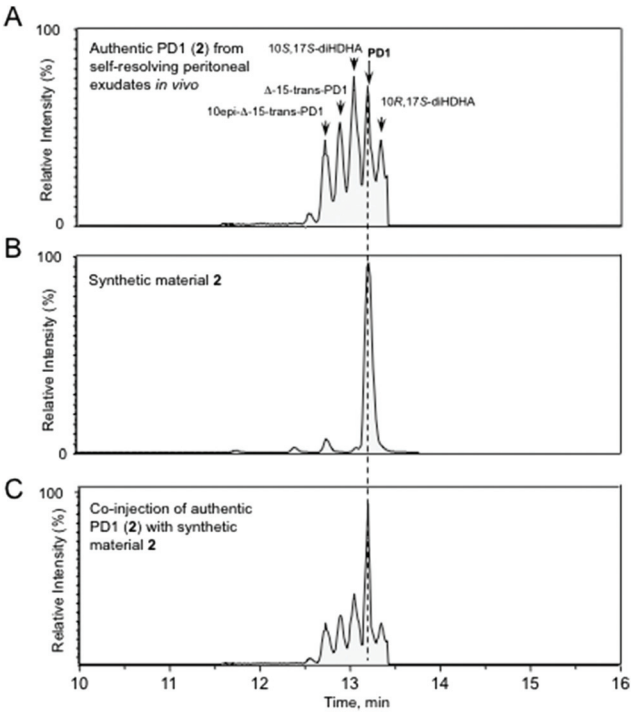


Fig. 3 HPLC chromatograms obtained from the matching experiments. Authentic protectin D1 (**2**) from self-resolving peritoneal inflammatory exudates matched synthetic material protectin D1 (**2**). Selected ion chromatograms (*m/z* 359–153) depicting (A) authentic protectin D1 (**2**), marked as PD1, 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).

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, $CHCl_3$); ^{1}H 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, $CHCl_3$); ^{1}H 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, $CHCl_3$); ^{1}H 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); ^{1}H 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*-butyldi-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); ^{1}H 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-dihydroxydo-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]

Acknowledgements

The Norwegian Research Council (KOSK II) and The School of Pharmacy, University of Oslo are gratefully acknowledged for Ph.D.-scholarships to M. A. and J. E. T., respectively. T. V. H. is grateful for a Leiv Eriksson travel grant from The Norwegian Research Council. J. D. is supported by the National Institutes of Health GM Grant PO1GM095467 (awarded to Charles N. Serhan). Fruitful discussions with Professor Charles N. Serhan, Brigham and Women's Hospital and Harvard Medical School are gratefully appreciated.

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