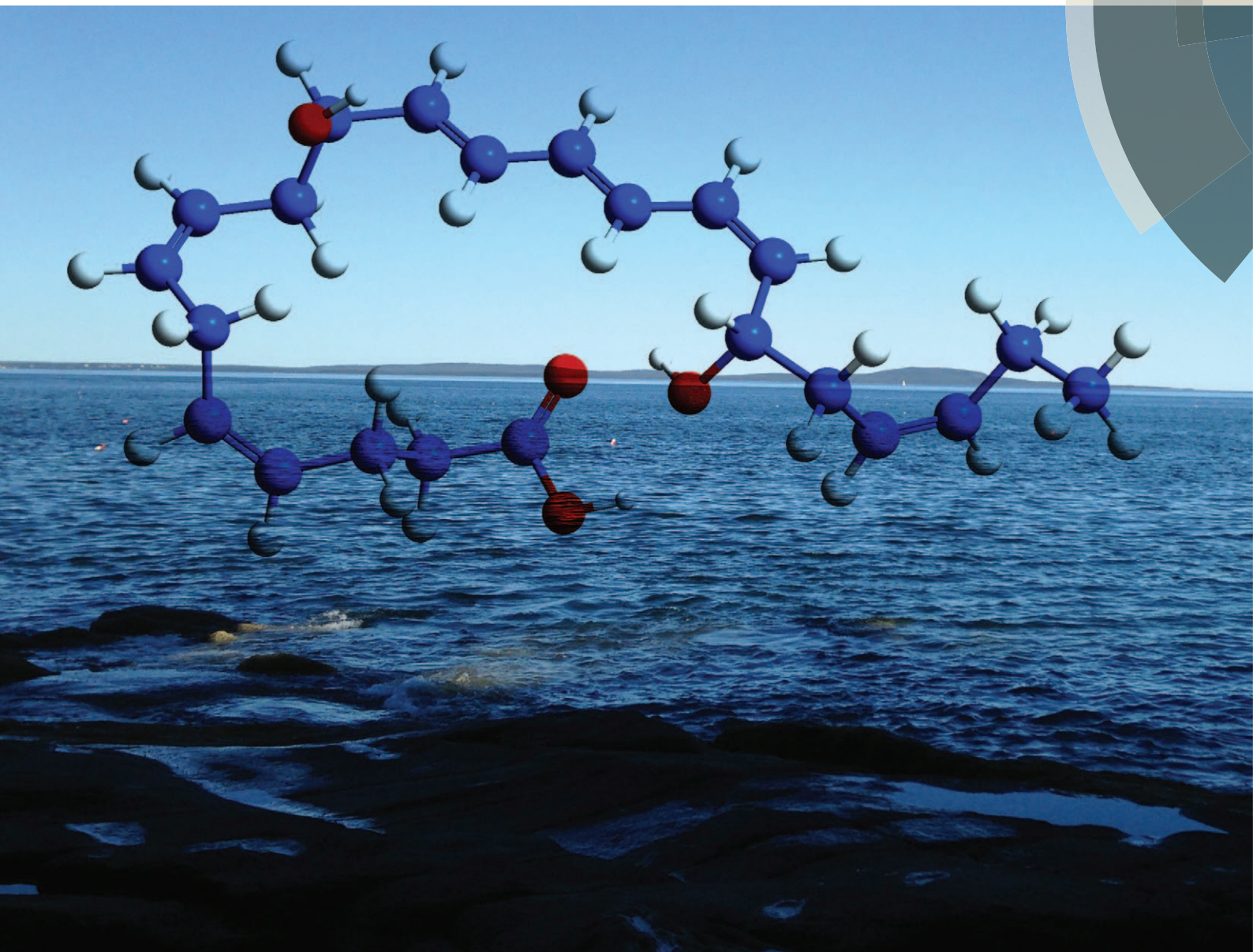


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**PAPER**

T. V. Hansen *et al.*

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

## 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.<sup>1</sup> 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**),<sup>2</sup> maresin 1,<sup>3</sup> resolvin D1 and resolvin D3.<sup>2a,4</sup> All of these compounds have enabled new research areas related to many disease states associated with inflammation.<sup>5</sup> 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).<sup>6</sup>

This compound has been reported to exhibit strong *in vivo* protective activity in several inflammatory<sup>6</sup> as well as many other disease models.<sup>7–10</sup> For example, the oxygenated polyunsaturated fatty acid **2** protects the retina and the brain from oxidative stress with very potent agonist activities.<sup>7</sup> It is noteworthy that **2** was observed to be several orders of magnitude more potent *in vivo* than its precursor DHA.<sup>2c</sup> Moreover, additional biological effects have recently been reported for this C22-oxygenated metabolite.<sup>11</sup> Hence, protectin D1 (**2**) is

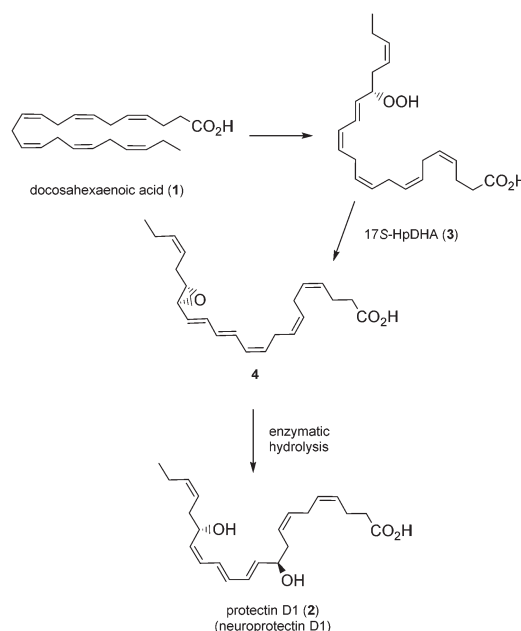


Fig. 1 Biosynthesis of protectin D1 (**2**).

very interesting as a lead compound for the development of potential new anti-inflammatory drugs.<sup>12</sup> The prefix *neuro* is added when this oxygenated PUFA is formed by neural tissues.<sup>2a</sup> As of today, two syntheses of protectin D1 (**2**) have appeared.<sup>6,13</sup> In connection with our interest in the synthesis of biologically active PUFA-derived natural products,<sup>14</sup> 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<sup>2–4</sup> 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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†Electronic supplementary information (ESI) available: Additional experimental procedures and characterization data, <sup>1</sup>H-, <sup>13</sup>C-NMR, HRMS, LC-MS/MS and UV/VIS spectra as well as chromatograms of HPLC analyses. See DOI: 10.1039/c3ob41902a



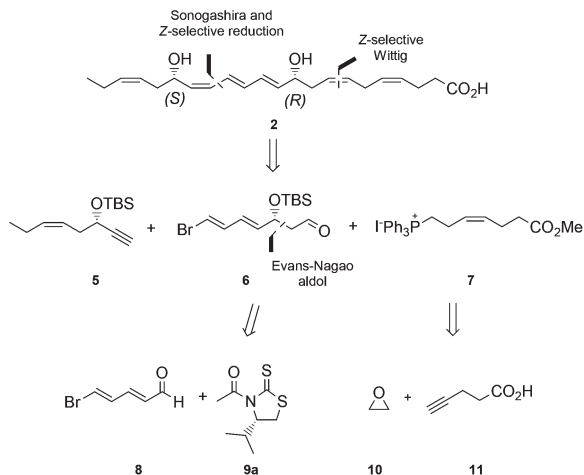


Fig. 2 Retrosynthetic analysis of protectin D1 (2).

## Results and discussion

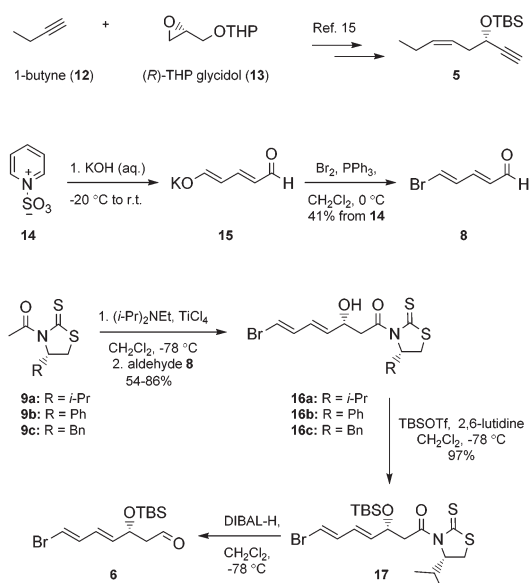
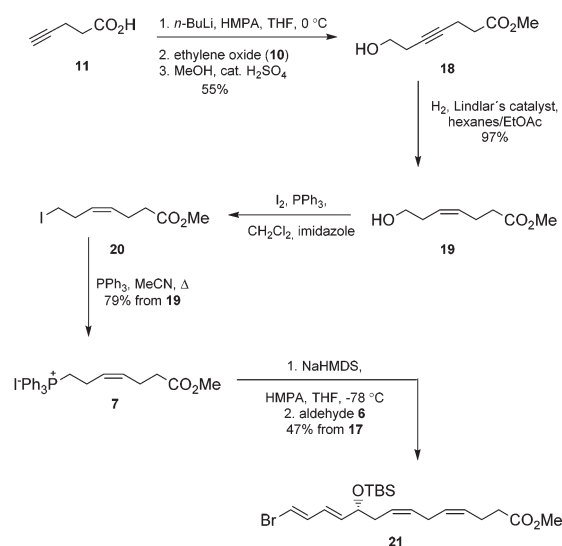
Our synthesis of **2** commenced with the preparation of **5**, essentially as previously reported,<sup>15</sup> from 1-butyne (**12**) and THP-protected (*R*)-glycidol **13** (Scheme 1).

Aldehyde **8** was prepared by a slightly modified and improved literature protocol.<sup>16</sup> Commercially available pyridinium-1-sulfonate (**14**) was treated with aqueous potassium hydroxide at  $-20\text{ }^{\circ}\text{C}$  to yield glutacetaldehyde potassium salt **15** that was transformed further with the  $\text{Br}_2/\text{PPh}_3$  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,<sup>17</sup> in an Evans-aldol<sup>18</sup> type reaction using conditions developed by Olivo and co-workers ( $\text{TiCl}_4$ ,  $\text{Et}(\text{i-Pr})_2\text{N}$ ,  $\text{CH}_2\text{Cl}_2$ ,  $-78\text{ }^{\circ}\text{C}$ ).<sup>19</sup> This smoothly produced the intermediate **16a** in a 15.3:1 diastereomeric

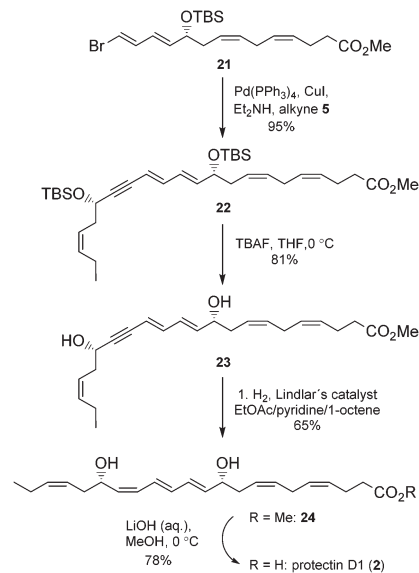
ratio as determined by HPLC and  $^1\text{H}$  NMR analyses. We also investigated reactions using thiazolidinones **9b** and **9c**, with the phenyl and the benzyl group, respectively, which afforded **16b** and **16c** with lower diastereoselectivity (4.5:1 and 9.8:1). Purification by chromatography yielded diastereomeric pure **16a** in 86% isolated yield. Protection of the alcohol functionality in **16a** to compound **17** was achieved using standard conditions.<sup>20</sup> Then DIBAL-H-reduction of **17** in  $\text{CH}_2\text{Cl}_2$  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,<sup>21</sup> 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  $\text{H}_2\text{SO}_4$ ), 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  $^1\text{H}$  NMR analyses. Then an Appel reaction<sup>22</sup> provided the iodide **20** which was treated with  $\text{PPh}_3$  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 reaction<sup>23</sup> 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 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**.<sup>24</sup> 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<sup>25</sup> produced triene **24** with high stereoselectivity, while the diimide reduction<sup>26</sup> or the standard Lindlar hydrogenation reaction<sup>27</sup> of **23** failed to give a high conversion to **24**. The Boland reduction<sup>28</sup> 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<sub>2</sub>PO<sub>4</sub>) 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.<sup>6</sup> 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.<sup>29</sup> 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.<sup>30</sup> 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<sub>2</sub>O,  $m/z$  323 = M–H–2H<sub>2</sub>O,  $m/z$  315 = M–H–CO<sub>2</sub>,  $m/z$  297 = M–H–H<sub>2</sub>O–CO<sub>2</sub>,  $m/z$  279 = M–H–2H<sub>2</sub>O–CO<sub>2</sub>,  $m/z$  243 = 261–H<sub>2</sub>O,  $m/z$  199 = 261–H<sub>2</sub>O–CO<sub>2</sub>,  $m/z$  188 = 206–H<sub>2</sub>O,  $m/z$  135 = 153–H<sub>2</sub>O,  $m/z$  121 = 181–H<sub>2</sub>O–CO<sub>2</sub>,  $m/z$  109 = 153–CO<sub>2</sub> (see ESI†). Similar results were also obtained when synthetic ester **24** was hydrolysed to the acid **2** and compared with authentic protectin

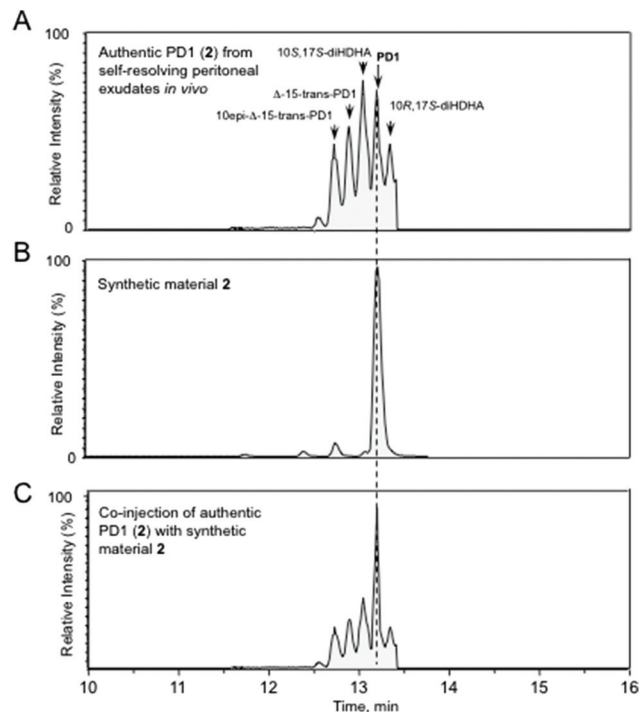


Fig. 3 HPLC chromatograms obtained from the matching experiments. Authentic protectin D1 (2) from self-resolving peritoneal inflammatory exudates matched synthetic material protectin D1 (2). Selected ion chromatograms ( $m/z$  359–153) depicting (A) authentic protectin D1 (2), marked as PD1, obtained 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,<sup>6</sup> 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,<sup>13b</sup> but not with the spectra published by others.<sup>13a</sup>

## 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.*<sup>19b</sup> All spectroscopic and physical data were in full agreement with those reported in the literature.<sup>19b</sup>  $[\alpha]_{\text{D}}^{20} = 31.5$  ( $c = 0.2$ ,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  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);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  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-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.<sup>19a</sup> The diastereomeric ratio (15.3:1) on the crude product was determined by HPLC analysis (Eclipse XDB-C18,  $\text{MeOH-H}_2\text{O}$  70:30, 1.0 mL  $\text{min}^{-1}$ ,  $t_{\text{r}}(\text{minor}) = 8.65$  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.<sup>19a</sup>  $[\alpha]_{\text{D}}^{20} = 271.3$  ( $c = 0.13$ ,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  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);  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  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,<sup>31</sup> the alcohol **16a** was protected with a TBS-group. Yield: 4.2 g (97%);  $[\alpha]_{\text{D}}^{20} = 263$  ( $c = 0.5$ ,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  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);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  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).  $\text{Et}_2\text{O}$  (15 mL) was added and the phases were separated. The aqueous phase was extracted with  $\text{Et}_2\text{O}$  ( $2 \times 15$  mL) and the combined organic layers were dried ( $\text{Na}_2\text{SO}_4$ ), before it was concentrated *in vacuo*. The crude product was purified by column chromatography on silica (hexanes– $\text{EtOAc}$  95:5) to afford the title compound **21** as a yellow oil. Yield: 217 mg (47% for two steps starting from **17**); TLC (hexanes– $\text{EtOAc}$  95:5, CAM stain):  $R_{\text{f}} = 0.29$ ;  $[\alpha]_{\text{D}}^{20} = -9.4$  ( $c = 0.1$ ,  $\text{MeOH}$ );  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  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);  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  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  $\text{ES}^+$ ): 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  $\text{Et}_2\text{NH}$  (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.  $\text{CuI}$  (5 mg, 0.03 mmol, 5 mol%) in a minimum amount of  $\text{Et}_2\text{NH}$  was added followed by dropwise addition of alkyne **5** (117 mg, 0.49 mmol, 1.0 equiv.) in  $\text{Et}_3\text{N}$  (1.0 mL). After 20 h of stirring at ambient temperature, the reaction was quenched by the addition of saturated  $\text{NH}_4\text{Cl}$  (15 mL).  $\text{Et}_2\text{O}$  (15 mL) was added and the phases were separated. The aqueous phase was extracted with  $\text{Et}_2\text{O}$  ( $2 \times 15$  mL) and the combined organic layers were dried ( $\text{Na}_2\text{SO}_4$ ), before being concentrated *in vacuo*. The crude product was purified by column chromatography on silica (hexanes– $\text{EtOAc}$  95:5) to afford the title compound **22** as a pale yellow oil. Yield: 278 mg (95%); TLC (hexanes– $\text{EtOAc}$  9:1, CAM stain):  $R_{\text{f}} = 0.44$ ;  $[\alpha]_{\text{D}}^{20} = -15.5$  ( $c = 0.20$ ,  $\text{MeOH}$ );  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  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);  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  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  $\text{ES}^+$ ): 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<sub>2</sub>SO<sub>4</sub>) 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*<sub>f</sub> = 0.19; [ $\alpha$ ]<sub>D</sub><sup>20</sup> = −9.2 (*c* = 0.3, MeOH); <sup>1</sup>H NMR (400 MHz, MeOD-*d*<sub>4</sub>)  $\delta$  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); <sup>13</sup>C NMR (101 MHz, MeOD-*d*<sub>4</sub>)  $\delta$  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<sup>+</sup>): Exact mass calculated for C<sub>23</sub>H<sub>32</sub>O<sub>4</sub>Na [*M* + Na]<sup>+</sup>: 395.2198, found 395.2206.

**Methyl (4Z,7Z,10R,11E,13E,15Z,17S,19Z)-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<sub>2</sub>O 75:25, 1.0 mL min<sup>−1</sup>): *t*<sub>r</sub>(minor) = 12.62 min, and *t*<sub>r</sub>(major) = 9.07 min. Yield: 19.5 mg (65%); TLC (hexanes–EtOAc 6:4, CAM stain): *R*<sub>f</sub> = 0.19; [ $\alpha$ ]<sub>D</sub><sup>20</sup> = −22.2 (*c* = 0.4, MeOH); UV (MeOH)  $\lambda$ <sub>max</sub> 262, 271, 282 nm. <sup>1</sup>H NMR (400 MHz, MeOD-*d*<sub>4</sub>)  $\delta$  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); <sup>13</sup>C NMR (101 MHz, MeOD-*d*<sub>4</sub>)  $\delta$  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<sup>+</sup>): Exact mass calculated for C<sub>23</sub>H<sub>34</sub>O<sub>4</sub>Na [*M* + Na]<sup>+</sup>: 397.2354, found 397.2365. All spectroscopic and physical data were in agreement with those reported in the literature.<sup>13b</sup>

**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<sub>2</sub>PO<sub>4</sub> (4.0 mL) was added. Next, NaCl (10.0 g) was added followed by EtOAc (50 mL). The organic phase was decanted, dried (Na<sub>2</sub>SO<sub>4</sub>), 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<sub>2</sub>O, 7:3, 1.0 mL min<sup>−1</sup>): *t*<sub>r</sub>(minor) = 9.97 min and *t*<sub>r</sub>(major) = 10.68 min; TLC (hexanes–EtOAc 6:4, CAM stain): *R*<sub>f</sub> = 0.03; [ $\alpha$ ]<sub>D</sub><sup>20</sup> = −24.0 (*c* = 0.3, MeOH); UV (MeOH)  $\lambda$ <sub>max</sub> 262, 271, 282 nm. IR (neat)  $\nu$  = 3316, 3012, 2961, 2930, 1713, 1557 cm<sup>−1</sup>; <sup>1</sup>H NMR (300 MHz, MeOH-*d*<sub>4</sub>)  $\delta$  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); <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>)  $\delta$  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<sup>−</sup>): Exact mass calculated for C<sub>22</sub>H<sub>31</sub>O<sub>4</sub> [*M* − H]<sup>−</sup>: 359.2222, found 359.2213. All spectroscopic and physical data were in agreement with those reported in the literature.<sup>13b</sup>

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