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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.

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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.^{2*a*,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 poly-unsaturated fatty acid 2 protects the retina and the brain from oxidative stress with very potent agonist activities.⁷ It is note-worthy 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 °C to yield glutaconaldehyde potassium salt **15** that was transformed further with the Br₂/PPh₃ 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 Evansaldol¹⁸ type reaction using conditions developed by Olivo and co-workers (TiCl₄, Et(i-Pr)₂N, CH₂Cl₂, -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 CH₂Cl₂ 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 H₂SO₄), 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 PPh₃ 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 reaction²³ at ambient temperature in the presence of Pd-(PPh₃)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.





CO₂Me

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 (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 ($T_{\rm R}$ = 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-2 H_2O , m/z 315 = M-H- CO_2 , m/z 297 = M-H- H_2O-CO_2 , m/z 279 = M-H-2 H_2O-CO_2 , m/z 243 = 261- H_2O , m/z199 = 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 (10⁵ 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 multimg 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} $[a]_D^{20} = 31.5$ (c = 0.2, CHCl₃); ¹H NMR (300 MHz, CDCl₃) 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); ¹³C NMR (75 MHz, CDCl₃) 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-H₂O 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.^{19*a*} $[\alpha]_{D}^{20} = 271.3 \ (c = 0.13, \text{CHCl}_3); {}^{1}\text{H NMR} \ (300 \text{ MHz},$ $CDCl_3$) 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); ¹³C NMR (101 MHz, CDCl₃) 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, CHCl₃); ¹H NMR (300 MHz, CDCl₃) 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); ¹³C NMR (75 MHz, CDCl₃) 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*-butyldimethyl-silyl)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): $R_{\rm f} = 0.29$; $[\alpha]_{\rm D}^{20} = -9.4$ (c = 0.1, MeOH); ¹H NMR (400 MHz, CDCl₃) δ 6.68 (dd, I = 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); ¹³C NMR (101 MHz, CDCl₃) 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 Et₂NH (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 Et₂NH was added followed by dropwise addition of alkyne 5 (117 mg, 0.49 mmol, 1.0 equiv.) in Et₃N (1.0 mL). After 20 h of stirring at ambient temperature, the reaction was quenched by the addition of saturated NH₄Cl (15 mL). Et₂O (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 (Na₂SO₄), 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): $R_{\rm f} = 0.44$; $[\alpha]_{\rm D}^{20} = -15.5$ (c = 0.20, MeOH); ¹H NMR (400 MHz, CDCl₃) 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); ¹³C NMR (101 MHz, CDCl₃) 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 guenched 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 \times 30 mL) and the combined organic layer was dried (Na₂SO₄) 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): $R_{\rm f} = 0.19$; $[\alpha]_{\rm D}^{20} = -9.2$ (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); ¹³C 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 (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 EtOAcpyridine-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): $R_{\rm f} = 0.19$; $[\alpha]_{\rm D}^{20} = -22.2$ (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); ¹³C 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 NaH₂PO₄ (4.0 mL) was added. Next, NaCl (10.0 g) was added followed by EtOAc (50 ml). The organic phase was decanted, dried (Na₂SO₄), 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): $t_r(\text{minor}) = 9.97$ min and $t_r(\text{major}) =$ 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); ¹³C 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 $C_{22}H_{31}O_4 [M - H]^-$: 359.2222, found 359.2213. All spectroscopic and physical data were in agreement with those reported in the literature.^{13b}

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