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Total synthesis of pro-resolving and tissue-regenerative Protectin sulfido-conjugates

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ABSTRACT

The stereospecific total synthesis of the pro-resolving and tissue-regenerative Protectin sulfido-conjugates: 16*R*,17*S*-PCTR1, 16*R*,17*S*-PCTR2, and 16*R*,17*S*-PCTR3, derived from docosahexaenoic acid, has been achieved. The key intermediate 16*S*,17*S*-epoxy-Protectin methyl ester was synthesized using the Sharpless catalytic asymmetric epoxidation to generate the chiral centers at C16 and C17. A Cs_2CO_3 promoted coupling provided the skipped diyne intermediate. Wittig reactions and epoxide opening with glutathione, L-cysteinylglycine, and L-cysteine methyl ester hydrochloride, respectively, were the key steps in the synthesis.

It has been extensively described in the literature that ω-3 polyunsaturated fatty acids mainly eicosapentaenoic and docosahexaenoic acids have anti-inflammatory activities and decrease the seriousness of infection.[1,2] These fatty acids have been investigated in a variety of diseases including rheumatoid arthritis, asthma, and cardiovascular diseases. Their effects on neutrophil function and leukotriene generation in humans have been described.[3–5] The molecules responsible for these beneficial actions were discovered by Serhan and collaborators to be lipoxygenase derivatives of these fatty acids. In their pioneering experiments they identified in the exudates of self-resolving inflammation several polyhydroxylated metabolites of eicosapentaenoic and docosahexaenoic acids with powerful anti-inflammatory and pro-resolution activities: Resolvins, Protectins, and Maresins, collectively named specialized pro-resolving mediators (SPMs).[6–12] A new class of SPMs derived from docosapentaenoic acid has just been reported.[13]

Recently, Serhan and collaborators investigated self-resolving exudates in *Escherichia coli* infected mice, human spleen, and blood of sepsis patients, and discovered the novel anti-inflammatory, pro-resolving, and tissue regenerative sulfido-conjugates of Maresin, Protectin, and Resolvin.[14,15] These compounds promote the pathogen clearance and could provide a new way to treat infections. The proposed biosynthesis of the Protectin sulfido-conjugates is based on the identified precursor and its products formed as shown in Figure 1. Docosahexaenoic acid is converted to the 17*S*-hydroperoxide intermediate that undergoes epoxide formation to produce 16*S*,17*S*-epoxy-Protectin. Enzymatic hydrolysis gave Protectin D1 whereas glutathione *S*-transferase gives the 16,17*S*-Protectin conjugate in tissue regeneration (PCTR1). The authors pointed out that it is most likely that the stereochemistry at C16 is *R* similar as described for the peptido-leukotrienes via SN_2 epoxide opening by glutathione.[16–19] PCTR1 is further metabolized by a γ-glutamyl transpeptidase to PCTR2 and finally by a dipeptidase to PCTR3.[15]

Due to the limited availability from natural sources these sulfido-conjugates have to be prepared by total synthesis to make them available for further pharmacological evaluation. So far a total synthesis of the Protectin sulfido-conjugates has not been reported. We recently reported the total syntheses of the Maresin sulfido-conjugates.[20]

In this Letter we wish to report the first total synthesis of the 17-series sulfido-conjugated mediators 16*R*,17*S*-PCTR1 [(4*Z*,7*Z*,10*Z*,12*E*,14*E*,16*R*,17*S*,19*Z*)-16-glutathionyl-17-hydroxy-4,7,10,12,14,19-docosahexaenoic acid (**1**)], 16*R*,17*S*-PCTR2 [(4*Z*,7*Z*,10*Z*,12*E*,14*E*,16*R*,17*S*,19*Z*)-16-cysteinylglycinyl-17-hydroxy-4,7,10,12,14,19-docosahexaenoic acid (**2**)], and 16*R*,17*S*-PCTR3 [(4*Z*,7*Z*,10*Z*,12*E*,14*E*,16*R*,17*S*,19*Z*)-16-cysteinyl-17-hydroxy-4,7,10,12,14,19-docosahexaenoic acid (**3**)]. As shown in the retrosynthetic approach (Fig. 2) **1**, **2**, and **3** have been prepared from the chiral

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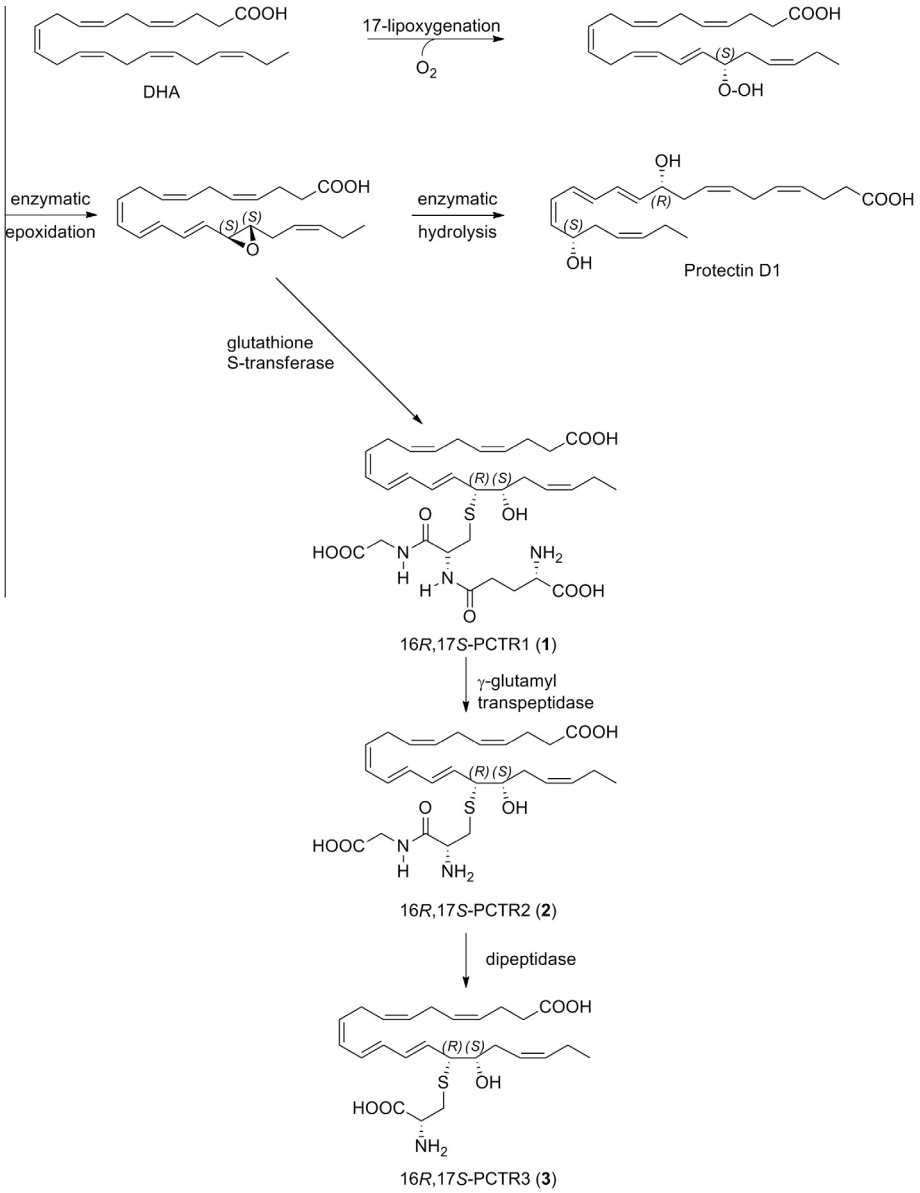


Figure 1. Biosynthesis of Protectin D1 and proposed biosynthesis of 16*R*,17*S*-PCTR1, 16*R*,17*S*-PCTR2 and 16*R*,17*S*-PCTR3 from DHA.[15]

16*S*,17*S*-epoxy-Protectin methyl ester (**4**) via epoxide opening with glutathione, L-cysteinylglycine, and L-cysteine methyl ester hydrochloride, respectively. The key intermediate **4** has been prepared from intermediates **5**, **6**, and **7**. The chiral centers of the epoxy-aldehyde **7** were generated using the Sharpless catalytic asymmetric epoxidation. Phosphonium iodide **5** has been synthesized from the alkynes **8** and **9**.

The synthesis of **5** is outlined in Scheme 1. 5-[(Tetrahydro-2H-pyran-2-yl)oxy]-2-pentyn-1-ol (**9**) was converted to the iodide **10** with triphenylphosphine, imidazole, and iodine.[21] Cross coupling of iodide **10** with 4-pentynoic acid methyl ester (**8**)[22] in the presence of CuI, Cs_2CO_3, and TBAI in DMF gave the skipped diyne **11** in 77% yield.[23] The semi-hydrogenation of **11** using Lindlar catalyst turned out to be challenging. It was difficult to avoid partial and over-reduction. The best results were achieved using Lindlar catalyst in hexane/ethyl acetate in the presence of pyridine to give *Z*,*Z*-skipped diene **12** in 40% isolated yield. Other base modifiers were less effective.[24] Cleavage of the THP group in **12** was achieved in 92% yield with PPTS in methanol to give **13**. Compound **13** was transformed to the iodide **14** and then converted to the phosphonium salt **5** with 1.1 equiv of triphenylphosphine in acetonitrile at 75 °C for 36 h in 93% yield. The excess of triphenylphosphine was removed by extracting the acetonitrile phase with hexane.

The synthesis of 16*S*,17*S*-epoxy-Protectin methyl ester (**4**) is described in Scheme 2. Sharpless cat. AE of (2*E*)-2-octen-5-yn-1-ol (**15**) gave the chiral epoxy alcohol **16** as previously described.[25] The crystalline nature of **16** allowed to obtain this key intermediate with >98% ee after recrystallization. Lindlar reduction of **16** in hexane/ethyl acetate in the presence of triethylamine produced **17** that was oxidized using the Dess–Martin periodinane reagent in CH_2Cl_2 affording the epoxy aldehyde **7** in 93% yield.[26] The four-carbon homologation of **7** with recrystallized (2*E*)-4-triphenylphosphoranylidene-2-butenal (**6**) in CH_2Cl_2 at rt for 17 h followed by treatment with catalytic iodine in benzene afforded the *E*,*E*-dienal **18**.[27,28] Next, *Z*-selective Wittig reaction of **18** with the ylide prepared in situ from the phosphonium iodide **5** with KHMDS at −78 °C in THF afforded the key epoxy ester **4** in 65% isolated yield after alkaline quench and flash chromatography purification in the presence of triethylamine. Compound **4** was characterized by ^{1}H NMR, ^{13}C NMR, COSY, HSQC, and UV.[29] The geometry of the

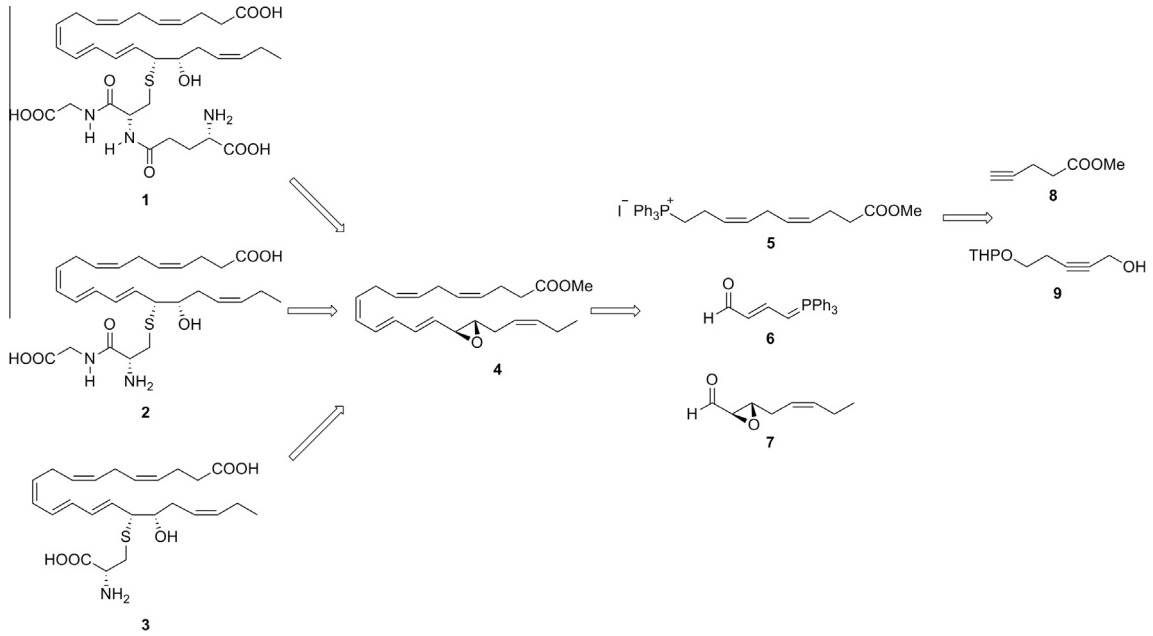
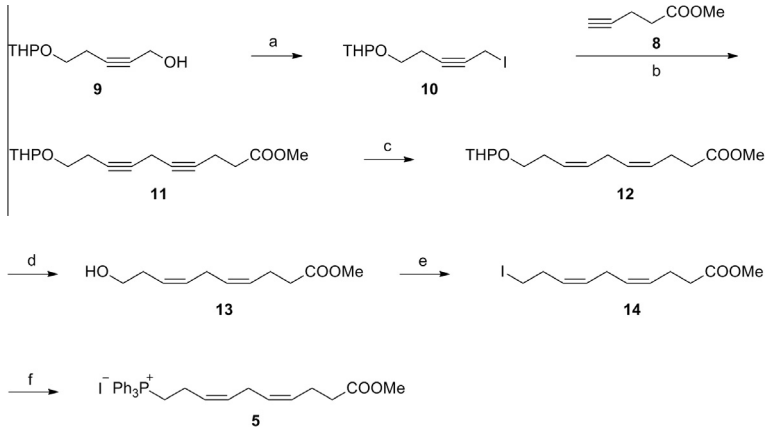
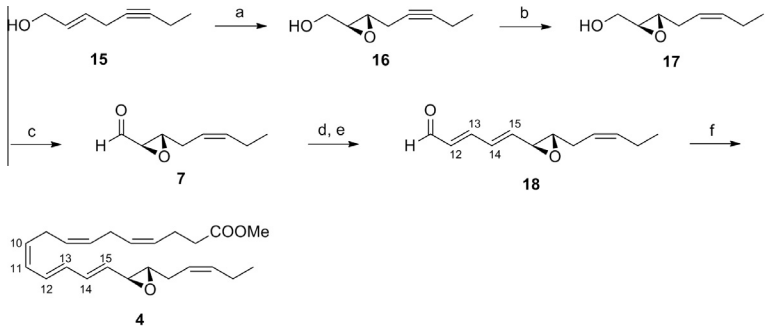


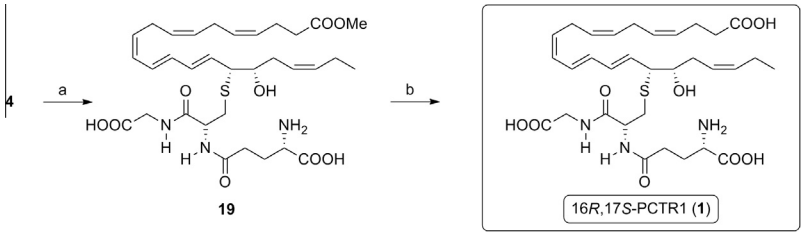
Figure 2. Retrosynthetic approach to 16*R*,17*S*-PCTR1, 16*R*,17*S*-PCTR2 and 16*R*,17*S*-PCTR3.



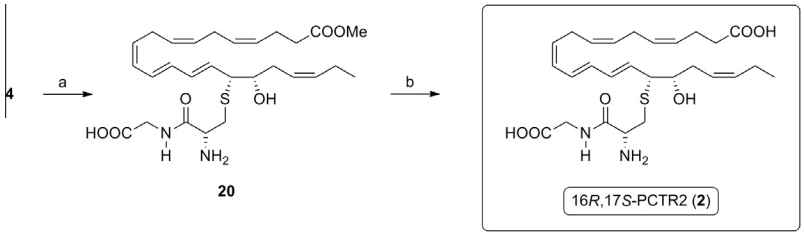
Scheme 1. Reagents and conditions: (a) I_2, Ph_3P, imidazole, THF, 75%; (b) Cs_2CO_3, CuI, TBAI, DMF, rt, 77%; (c) H_2, Lindlar cat., pyridine, EtOAc, hexane, 40%; (d) PPTS, CH_3OH, rt, 92%; (e) I_2, Ph_3P, imidazole, *i*-Pr_2EtN, CH_3CN, ether; (f) Ph_3P, CH_3CN, 75 °C, 93% (over two steps).



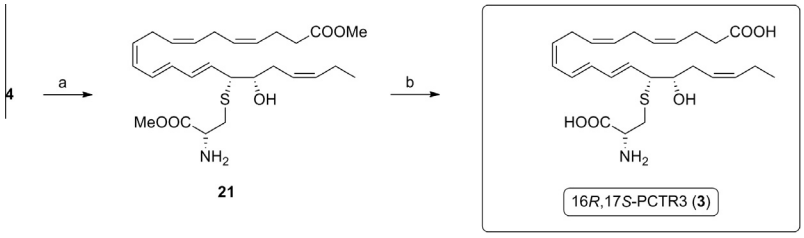
Scheme 2. Reagents and conditions: (a) Ref. 25; (b) H_2, Lindlar cat., Et_3N, EtOAc, hexane, 74%; (c) Dess–Martin periodinane, $NaHCO_3$, CH_2Cl_2, rt, 93%; (d) **6**, CH_2Cl_2, rt, 46%; (e) cat. I_2, benzene, rt, 84%; (f) **5**, KHMDS, THF, −78 °C to −10 °C, 65%.



Scheme 3. Reagents and conditions: (a) Glutathione, Et_3N, H_2O, CH_3OH, rt; (b) 1 N LiOH, H_2O, 0 °C to rt, 55% (over two steps).



Scheme 4. Reagents and conditions: (a) L-cysteinylglycine, Et_3N, H_2O, CH_3OH, rt; (b) 1 N LiOH, H_2O, 0 °C to rt, 50% (over two steps).



Scheme 5. Reagents and conditions: (a) L-cysteine methyl ester hydrochloride, Et_3N, H_2O, CH_3OH, rt, 67%; (b) 1 N LiOH, H_2O, CH_3OH, 0 °C to rt, 49%.

12*E*,14*E*-diene unit in **18** ($J_{12,13}$ = 15.3 and $J_{14,15}$ = 15.3) and the 10*Z*,12*E*,14*E*-triene in **4** ($J_{10,11}$ = 11.4, $J_{12,13}$ = 15.0 and $J_{14,15}$ = 15.3) was confirmed by the ^1H–^1H coupling constants.[29]

The conversion of epoxy ester **4** to 16*R*,17*S*-PCTR1 (**1**) was achieved in two steps as described in Scheme 3.[20,30,31] Reaction of **4** with 3 equiv of glutathione in CH_3OH/triethylamine/H_2O gave the monomethyl ester **19**. Mild hydrolysis of crude **19** with 1 N LiOH in H_2O for 30 min afforded 16*R*,17*S*-PCTR1 (**1**) that was purified by HPLC [Zorbax SB-C18 250 × 21.2 mm, 280 nm, CH_3OH/H_2O (0.1% NH_4OAc, pH 5.6, 0.05% EDTA disodium) 65/35]. The fraction containing **1** was desalted using a reversed phase C-18 cartridge to give pure 16*R*,17*S*-PCTR1 (**1**). The ^1H NMR, COSY, UV, and HPLC/UV/MS analyses were consistent with the structure of **1**.[29]

Compound **4** was reacted with L-cysteinylglycine to obtain compound **20** that was converted to 16*R*,17*S*-PCTR2 (**2**) using the same conditions as described for **1** (Scheme 4). 16*R*,17*S*-PCTR2 (**2**) was purified by HPLC [Zorbax SB-C18 250 × 21.2 mm, 280 nm, CH_3OH/H_2O (0.1% NH_4OAc, pH 5.6, 0.05% EDTA disodium) 65/35]. Compound **4** was reacted with L-cysteine methyl ester hydrochloride to obtain 16*R*,17*S*-PCTR3-dimehtyl ester (**21**)[32] that was converted to 16*R*,17*S*-PCTR3 (**3**) using similar conditions as described for **1** and **2** (Scheme 5). 16*R*,17*S*-PCTR3 (**3**) was purified by HPLC [Zorbax SB-C18 250 × 21.2 mm, 280 nm, CH_3OH/H_2O (0.1% NH_4OAc, pH 5.6, 0.05% EDTA disodium) 65/35]. The ^1H NMR, COSY, UV, and HPLC/UV/MS analyses were consistent with the structures of **2** and **3**.[29]

In summary, a concise total synthesis of 16*R*,17*S*-PCTR1, 16*R*,17*S*-PCTR2, and 16*R*,17*S*-PCTR3 has been achieved,[29] making these pro-resolving and tissue-regenerative lipid mediators from docosahexaenoic acid available for further biological and pharmacological testing. The synthesis of other specialized pro-resolving mediators (SPMs) will be reported in due course.

Acknowledgements

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References and notes

1. Dennis, E. A.; Norris, P. C. *Nat. Rev. Immunol.* **2015**, *15*, 511–523.
2. De Caterina, R. *N. Engl. J. Med.* **2011**, *364*, 2439–2450.
3. Lee, T. H.; Hoover, R. L.; Williams, J. D.; Sperling, R. I.; Ravalese, J.; Spur, B. W.; Robinson, D. R.; Corey, E. J.; Lewis, R. A.; Austen, K. F. *N. Engl. J. Med.* **1985**, *312*, 1217–1224.
4. Sperling, R. I.; Weinblatt, M.; Robin, J. L.; Ravalese, J.; Hoover, R. L.; House, F.; Coblyn, J. S.; Fraser, P. A.; Spur, B. W.; Robinson, D. R.; Lewis, R. A.; Austen, K. F. *Arthritis Rheum.* **1987**, *30*, 988–997.
5. Arm, J. P.; Horton, C. E.; Spur, B. W.; Mencia-Huerta, J. M.; Lee, T. H. *Am. Rev. Respir. Dis.* **1989**, *139*, 1395–1400.
6. Serhan, C. N. *Nature* **2014**, *510*, 92–101.
7. Serhan, C. N.; Chiang, N.; Van Dyke, T. E. *Nat. Rev. Immunol.* **2008**, *8*, 349–361.
8. Serhan, C. N.; Petasis, N. A. *Chem. Rev.* **2011**, *111*, 5922–5943.
9. Serhan, C. N.; Hong, S.; Gronert, K.; Colgan, S. P.; Devchand, P. R.; Mirick, G.; Moussignac, R. L. *J. Exp. Med.* **2002**, *196*, 1025–1037.
10. Serhan, C. N.; Yang, R.; Martinod, K.; Kasuga, K.; Pillai, P. S.; Porter, T. F.; Oh, S. F.; Spite, M. *J. Exp. Med.* **2009**, *206*, 15–23.
11. Chiang, N.; Fredman, G.; Backhed, F.; Oh, S. F.; Vickery, T.; Schmidt, B. A.; Serhan, C. N. *Nature* **2012**, *484*, 524–528.
12. Xu, Z. Z.; Zhang, L.; Liu, T.; Park, J. Y.; Berta, T.; Yang, R.; Serhan, C. N.; Ji, R. R. *Nat. Med.* **2010**, *16*, 592–597.
13. Dalli, J.; Chiang, N.; Serhan, C. N. *Nat. Med.* **2015**, *21*, 1071–1075.
14. Dalli, J.; Chiang, N.; Serhan, C. N. *Proc. Natl. Acad. Sci. U.S.A.* **2014**, *111*, E4753–E4761.
15. Dalli, J.; Ramon, S.; Norris, P. C.; Colas, R. A.; Serhan, C. N. *FASEB J.* **2015**, *29*, 2120–2136.

16. Corey, E. J.; Clark, D. A.; Goto, G.; Marfat, A.; Mioskowski, C.; Samuelsson, B.; Hammerstrom, S. *J. Am. Chem. Soc.* **1980**, *102*, 1436–1439.
17. Corey, E. J.; Marfat, A.; Goto, G. *J. Am. Chem. Soc.* **1980**, *102*, 6608–6609.
18. Rokach, J.; Adams, J. *Acc. Chem. Res.* **1985**, *18*, 87–93.
19. Yoshimoto, T.; Soberman, R. J.; Spur, B. W.; Austen, K. F. *J. Clin. Invest.* **1988**, *81*, 866–871.
20. Rodriguez, A. R.; Spur, B. W. *Tetrahedron Lett.* **2015**, *56*, 3936–3940.
21. Itoh, S.; Kuwahara, S.; Hasegawa, M.; Kodama, O. *Biosci. Biotechnol. Biochem.* **2002**, *66*, 1591–1596.
22. Rodriguez, A.; Nomen, M.; Spur, B. W.; Godfroid, J. J. *Tetrahedron Lett.* **1998**, *39*, 8563–8566.
23. Caruso, T.; Spinella, A. *Tetrahedron* **2003**, *59*, 7787–7790.
24. Oger, C.; Balas, L.; Durand, T.; Galano, J.-M. *Chem. Rev.* **2013**, *113*, 1313–1350.
25. Rodriguez, A. R.; Spur, B. W. *Tetrahedron Lett.* **2012**, *53*, 86–89.
26. Dess, D. B.; Martin, J. C. *J. Am. Chem. Soc.* **1991**, *113*, 7277–7287.
27. Berenguer, M. J.; Castells, J.; Galard, R. M.; Moreno-Manas, M. *Tetrahedron Lett.* **1971**, *12*, 495–496.
28. Ernest, I.; Main, A. J.; Menasse, R. *Tetrahedron Lett.* **1982**, *23*, 167–170.
29. Satisfactory spectroscopic data were obtained for all compounds. Selected physical data: Compound **10**: ^{1}H NMR ($CDCl_3$, 300 MHz): δ 4.7–4.6 (br t, J = 3.3 Hz, 1H), 3.9–3.8 (m, 1H), 3.8–3.7 (dt, J = 9.9, 6.9 Hz, 1H), 3.7–3.6 (t, J = 2.4 Hz, 2H), 3.6–3.5 (dt, J = 9.9, 6.9 Hz, 1H), 3.6–3.4 (m, 1H), 2.5 (tt, J = 6.9, 2.4 Hz, 2H), 1.9–1.5 (m, 6H); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 98.68, 83.33, 77.87, 65.23, 62.15, 30.49, 25.37, 20.59, 19.33, −17.33. Compound **11**: ^{1}H NMR ($CDCl_3$, 300 MHz): δ 4.6 (br t, J = 3.3 Hz, 1H), 3.9–3.8 (m, 1H), 3.8–3.7 (dt, J = 9.6, 7.2 Hz, 1H), 3.6 (s, 3H), 3.6–3.4 (dt, J = 9.6, 7.2 Hz, 1H), 3.5–3.4 (m, 1H), 3.1–3.0 (quint, J = 2.4 Hz, 2H), 2.6–2.4 (m, 6H), 1.8–1.4 (m, 6H); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 172.40, 98.68, 78.37, 77.28, 75.19, 75.09, 65.71, 62.17, 51.71, 33.32, 30.51, 25.38, 20.13, 19.38, 14.59, 9.70. Compound **12**: ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.5–5.3 (m, 4H), 4.6 (dd, J = 4.5, 2.7 Hz, 1H), 3.9–3.8 (m, 1H), 3.7 (dt, J = 9.6, 7.2 Hz, 1H), 3.6 (s, 3H), 3.5–3.4 (m, 1H), 3.4–3.3 (dt, J = 9.6, 7.2 Hz, 1H), 2.8 (br t, J = 5.7 Hz, 2H), 2.4–2.3 (m, 6H), 1.9–1.4 (m, 6H); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 173.54, 129.54, 129.37, 127.75, 126.20, 98.72, 66.89, 62.26, 51.53, 33.97, 30.66, 27.94, 25.64, 25.43, 22.75, 19.54. Compound **13**: ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.5–5.3 (m, 4H), 3.7 (s, 3H), 3.7–3.6 (m, 2H), 2.9–2.8 (br t, J = 6.4 Hz, 2H), 2.5–2.3 (m, 6H); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 173.65, 130.93, 129.17, 127.95, 125.77, 62.20, 51.59, 33.94, 30.85, 25.71, 22.81. Compound **14**: ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.6–5.3 (m, 4H), 3.7 (s, 3H), 3.2–3.1 (t, J = 7.2 Hz, 2H), 2.8 (br t, J = 6.0 Hz, 2H), 2.7–2.6 (br q, J = 7.2 Hz, 2H), 2.4–2.3 (m, 4H); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 173.50, 130.25, 128.82, 128.34, 128.14, 51.59, 33.92, 31.39, 25.73, 22.79, 5.26. Compound **5**: ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.9–7.7 (m, 15H), 5.7–5.6 (m, 1H), 5.5–5.3 (m, 1H), 5.3–5.2 (m, 2H), 3.9–3.8 (m, 2H), 3.6 (s, 3H), 2.6 (br t, J = 6.0 Hz, 2H), 2.6–2.4 (m, 2H), 2.4–2.1 (m, 4H); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 173.51, 135.12 (d, J = 2.8 Hz, 3C), 133.80 (d, J = 10.3 Hz, 6C), 130.52 (d, J = 12.0 Hz, 6C), 130.33, 128.44, 128.29, 126.39 (d, J = 14.3 Hz), 118.10 (d, J = 85.3 Hz, 3C), 51.58, 33.74, 25.57, 23.31 (d, J = 48.1 Hz), 22.75, 20.38 (d, J = 3.5 Hz); Compound **16**: ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.9 (dd, J = 12.6, 2.1 Hz, 1H), 3.6 (dd, J = 12.6, 4.2 Hz, 1H), 3.1 (m, 2H), 2.6–2.5 (ddt, J = 17.4, 4.2, 2.4 Hz, 1H), 2.5–2.4 (ddt, J = 17.4, 4.8, 2.4 Hz, 1H), 2.2–2.0 (qt, J = 7.5, 2.4 Hz, 2H), 2.1 (br s, 1H), 1.1 (t, J = 7.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 84.18, 73.27, 61.26, 57.83, 53.61, 21.67, 13.96, 12.31; $[\alpha]_D^{25}$ = −17.7 (c 0.73, $CHCl_3$). Compound **17**: ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.6–5.4 (dtt, J = 10.8, 7.2, 1.5 Hz, 1H), 5.4–5.2 (dtt, J = 10.8, 7.5, 1.5 Hz, 1H), 3.9 (dd, J = 12.6, 2.4 Hz, 1H), 3.6 (dd, J = 12.6, 4.2 Hz, 1H), 3.0–2.9 (m, 2H), 2.5–2.2 (m, 2H), 2.1–1.9 (m, 2H), 1.9–1.8 (br s, 1H), 0.95 (t, J = 7.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 134.92, 122.36, 61.75, 57.95, 55.29, 29.23, 20.66, 14.06; $[\alpha]_D^{25}$ = −27.0 (c 0.64, $CHCl_3$). Compound **7**: ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.0 (d, J = 6.3 Hz, 1H), 5.7–5.6 (dtt, J = 10.8, 7.2, 1.5 Hz, 1H), 5.4–5.2 (dtt, J = 10.8, 7.5, 1.5 Hz, 1H), 3.3 (td, J = 5.4, 2.1 Hz, 1H), 3.2 (dd, J = 6.3, 2.1 Hz, 1H), 2.6–2.4 (m, 2H), 2.2–2.0 (m, 2H), 1.0 (t, J = 7.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 198.29, 136.08, 120.79, 58.52, 55.98, 28.62, 20.70, 14.07. Compound **18**: ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.5 (d, J = 7.8 Hz, 1H), 7.1–7.0 (dd, J = 15.3, 11.1 Hz, 1H), 6.6 (dd, J = 15.3, 11.1 Hz, 1H), 6.2–6.1 (dd, J = 15.3, 7.8 Hz, 1H), 6.0–5.9 (dd, J = 15.3, 7.2 Hz, 1H), 5.6–5.5 (dtt, J = 10.8, 7.2, 1.5 Hz, 1H), 5.4–5.2 (dtt, J = 10.8, 7.5, 1.5 Hz, 1H), 3.2 (dd, J = 7.2, 2.1 Hz, 1H), 2.9 (td, J = 5.4, 2.1 Hz, 1H), 2.5–2.2 (m, 2H), 2.1–1.9 (m, 2H), 1.0 (t, J = 7.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 193.53, 149.99, 141.20, 135.25, 132.05, 130.82, 121.67, 60.72, 56.70, 29.41, 20.63, 14.09. Compound **4**: ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.6–6.5 (dd, J = 15.0, 11.4 Hz, 1H), 6.5–6.4 (dd, J = 15.3, 10.8 Hz, 1H), 6.2 (dd, J = 15.0, 10.8 Hz, 1H), 6.0 (br t, J = 11.4 Hz, 1H), 5.6–5.2 (m, 8H), 3.6 (s, 3H), 3.2–3.1 (dd, J = 8.2, 2.1 Hz, 1H), 3.0–2.9 (br t, J = 5.8 Hz, 2H), 2.9 (td, J = 5.4, 2.1 Hz, 1H), 2.9–2.8 (br t, J = 5.4 Hz, 2H), 2.5–2.2 (m, 6H), 2.0–2.1 (br quint, J = 7.5 Hz, 2H), 0.95 (t, J = 7.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 173.54, 134.89, 134.49, 131.58, 131.17, 130.23, 129.15, 128.69, 128.52 (2C), 127.98, 127.58, 122.27, 60.36, 58.06, 51.57, 33.97, 29.68, 26.23, 25.60, 22.80, 20.68, 14.18; UV (hexane) λ_{max} 271, 280, 292 nm. 16*R*,17*S*-PCTR1 (**1**): ^{1}H NMR (CD_3OD, 300 MHz): δ 6.6 (dd, J = 13.5, 11.1 Hz, 1H), 6.3–6.2 (m, 2H), 6.1–6.0 (t, J = 11.1 Hz, 1H), 5.8–5.6 (dd, J = 13.8, 10.2 Hz, 1H), 5.5–5.3 (m, 7H), 4.6–4.5 (dd, J = 9.3, 5.1 Hz, 1H), 3.9–3.7 (2d ABsystem, J = 17.4 Hz, 2H), 3.7 (td, J = 6.3, 3.9 Hz, 1H), 3.7–3.6 (t, J = 6.3 Hz, 1H), 3.4 (dd, J = 10.2, 3.9 Hz, 1H), 3.1–2.8 (m, 5H), 2.7 (dd, J = 13.8, 9.3 Hz, 1H), 2.6–2.5 (m, 2H), 2.4–2.2 (m, 5H), 2.2–2.1 (m, 3H), 2.0 (br quint, J = 7.5 Hz, 2H), 0.95 (t, J = 7.5 Hz, 3H); UV (CH_3OH) λ_{max} 272, 281, 292 nm; HPLC/UV: Zorbax SB-C18, 1.8 μm, 50 × 2.1 mm, 280 nm, CH_3OH/H_2O (0.1% formic acid) 35:65–70:30, 0.2 mL/min, t_R = 13.1 min; HPLC/MS (m/z): 648.2 $[M-H]^-$. 16*R*,17*S*-PCTR2 (**2**): ^{1}H NMR (CD_3OD, 300 MHz): δ 6.7–6.6 (dd, J = 14.4, 11.1 Hz, 1H), 6.4–6.3 (m, 2H), 6.1–6.0 (t, J = 11.1 Hz, 1H), 5.7 (dd, J = 14.1, 10.2 Hz, 1H), 5.6–5.3 (m, 7H), 4.0 (d, J = 17.4 Hz, 1H), 3.9–3.8 (t, J = 7.2 Hz, 1H), 3.8–3.7 (m, 1H), 3.6 (d, J = 17.4 Hz, 1H), 3.5–3.4 (dd, J = 10.2, 3.3 Hz, 1H), 3.1–2.8 (m, 5H), 2.8 (dd, J = 14.4, 7.2 Hz, 1H), 2.4–2.1 (m, 6H), 2.1–2.0 (br quint, J = 7.5 Hz, 2H), 0.95 (t, J = 7.5 Hz, 3H); UV (CH_3OH) λ_{max} 272, 281, 292 nm; HPLC/UV: Zorbax SB-C18, 1.8 μm, 50 × 2.1 mm, 280 nm, CH_3OH/H_2O (0.1% formic acid) 35:65–70:30, 0.2 mL/min, t_R = 11.9 min; HPLC/MS (m/z): 519.2 $[M-H]^-$. 16*R*,17*S*-PCTR3-dimethyl ester (**21**): ^{1}H NMR (CD_3OD, 300 MHz): δ 6.6 (dd, J = 13.5, 11.1 Hz, 1H), 6.4–6.2 (m, 2H), 6.1–6.0 (t, J = 11.1 Hz, 1H), 5.7–5.6 (dd, J = 13.8, 10.2 Hz, 1H), 5.5–5.3 (m, 7H), 3.8–3.7 (m, 1H), 3.7 (s, 3H), 3.6 (s, 3H), 3.6 (dd, J = 7.5, 5.1 Hz, 1H), 3.4–3.3 (dd, J = 10.2, 3.9 Hz, 1H), 3.0 (m, 2H), 2.9–2.8 (m, 3H), 2.7–2.6 (dd, J = 13.8, 7.5 Hz, 1H), 2.4–2.1 (m, 6H), 2.1–2.0 (br quint, J = 7.5 Hz, 2H), 0.95 (t, J = 7.5 Hz, 3H); ^{13}C NMR (CD_3OD, 75.5 MHz): δ 175.39, 175.28, 135.03, 134.87, 133.37, 131.35, 131.19, 130.24, 129.82, 129.46, 129.15, 129.06, 128.69, 125.63, 74.62, 55.15, 54.90, 52.72, 52.09, 36.09, 34.82, 34.27, 27.11, 26.50, 23.82, 21.78, 14.60; UV (CH_3OH) λ_{max} 272, 281, 292 nm. 16*R*,17*S*-PCTR3 (**3**): ^{1}H NMR (CD_3OD, 300 MHz): δ 6.6 (dd, J = 14.0, 11.1 Hz, 1H), 6.4–6.2 (m, 2H), 6.1–6.0 (t, J = 11.1 Hz, 1H), 5.7 (dd, J = 14.4, 10.2 Hz, 1H), 5.5–5.3 (m, 7H), 3.8–3.7 (ddd, J = 7.5, 6.0, 3.6 Hz, 1H), 3.7–3.6 (dd, J = 9.0, 3.6 Hz, 1H), 3.5 (dd, J = 10.2, 3.6 Hz, 1H), 3.1–2.8 (m, 6H), 2.5–2.1 (m, 6H), 2.1–2.0 (br quint, J = 7.5 Hz, 2H), 0.95 (t, J = 7.5 Hz, 3H); UV (CH_3OH) λ_{max} 272, 281, 292 nm; HPLC/UV: Zorbax SB-C18, 1.8 μm, 50 × 2.1 mm, 280 nm, CH_3OH/H_2O (0.1% formic acid) 35:65–70:30, 0.2 mL/min, t_R = 14.6 min; HPLC/MS (m/z): 462.2 $[M-H]^-$.
30. Cohen, N.; Banner, B. L.; Lopresti, R. J.; Wong, F.; Rosenberger, M.; Liu, Y.-Y.; Thom, E.; Liebman, A. A. *J. Am. Chem. Soc.* **1983**, *105*, 3661–3672.
31. Rodriguez, A.; Nomen, M.; Spur, B. W.; Godfroid, J.-J.; Lee, T. H. *Eur. J. Org. Chem.* **2000**, 2991–3000.
32. In a flame dried flask under argon compound **4** (8.6 mg, 0.024 mmol) was dissolved in Et_3N (100 μl) and treated with L-cysteine methyl ester hydrochloride (12.4 mg, 0.072 mmol) previously dissolved in $CH_3OH/Et_3N/H_2O$ (200 μl/100 μl/20 μl). The reaction mixture was stirred in the dark at rt for 5 h. Concentration followed by purification by flash chromatography ethyl acetate/hexane 80:20 (1% Et_3N) afforded 16*R*,17*S*-PCTR3-dimethyl ester (**21**) (7.9 mg, 67%).