124. Leiopathic Acid, a Novel Optically Active Hydroxydocosapentaenoic Acid, and Related Compounds, from the Black Coral *Leiopathes* sp. of Saint Paul Island (S. Indian Ocean)

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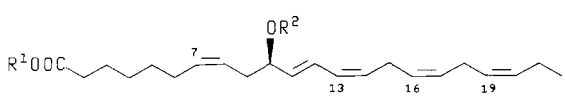
The antipatharian *Leiopathes* sp., collected around Saint Paul Island, is shown here to contain, in relatively high amounts, the novel fatty acid leiopathic acid (= (+)-(10*R*,7*Z*,11*E*,13*Z*,16*Z*,19*Z*)-10-hydroxy-7,11,13,16,19-docosapentaenoic acid; (+)-**1**), besides (+)-(8*R*,5*Z*,9*E*,11*Z*,14*Z*,17*Z*)-8-hydroxy-5,9,11,14,17-icosapentaenoic acid ((+)-**11**) and (+)-(8*R*,5*Z*,9*E*,11*Z*,14*Z*)-8-hydroxy-5,9,11,14-icosatetraenoic acid ((+)-**16**) and their ethyl esters (+)-**2**, (+)-**12**, and (+)-**17**.

1. Introduction. – Hydroperoxy- and hydroxy-substituted icosatetraenoic [1] and docosatetraenoic [2a] acids of mammals and pentaenoic analogues [2b][1]) have raised much interest as precursors of the physiologically important metabolites prostaglandins, thromboxanes, and their dihomo analogues, *via* lipooxygenase pathways [5].

We report here on a novel, optically active hydroxydocosapentaenoic acid, leiopathic acid ((+)-**1**), and its ethyl ester (+)-**2**, besides C_{20} unsaturated fatty acids, isolated from a hexacoral, the antipatharian *Leiopathes* sp. Not only the molecular structure but also the source of leiopathic acid are unusual as, in contrast with the rich productivity of octocorals and of other hexacorals, there is no previous record about natural products from members of the order Antipatharia, except for some work with primary metabolites of *Antipathes rhipidion* [6].

2. Docosapentaenoic Acid and Ester. – Extensive chromatography of extracts of the *Leiopathes* sp. lead to leiopathic acid ((+)-**1**) as an oil of the novel structure (+)-(10*R*,7*Z*,11*E*,13*Z*,16*Z*,19*Z*)-10-hydroxy-7,11,13,16,19-docosapentaenoic acid. Its ethyl ester (+)-**2** is isolated from the fraction of neutral lipids.



(+)-**1** $R^1 = R^2 = H$
(+)-**2** $R^1 = Et$, $R^2 = H$
(+)-**3** $R^1 = Me$, $R^2 = H$
4 $R^1 = Me$, $R^2 = Me_3Si$
(+)-**5** $R^1 = Me$, $R^2 = PhCO$
9 $R^1 = Me$, $R^2 = (S)$-MeCH(Ph)NHCO

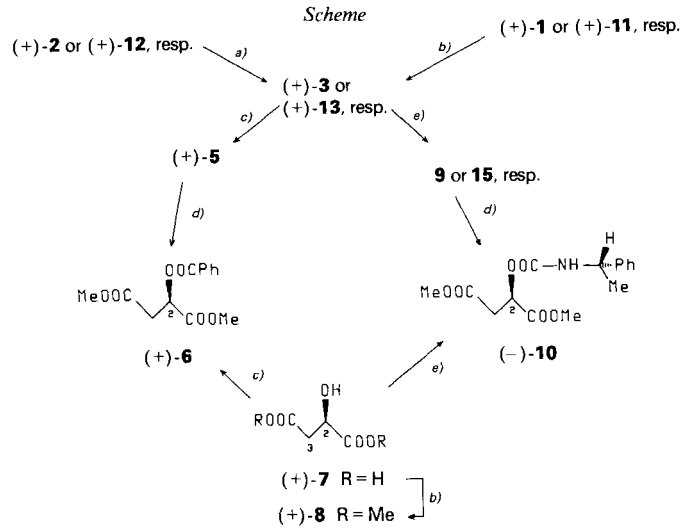
[1]) Also certain marine invertebrates contain enzymes capable of oxidizing arachidonic acid (= (all-*Z*)-5,8,11,14-icosatetraenoic acid). This occurs at either the C(8) position, such as with the stoloniferan coral *Clavularia viridis* [3], the gorgonian *Pseudoplexaura porosa* [4a], and starfish oocytes [4b], or at C(11) and C(12), such as with eggs of the sea urchin *Strongylocentrotus purpuratus* [4c]. Hydroxydocosahexaenoic acids are also known as products of oxidation of dietary docosahexanoic acid by rat-liver microsomes [4d].

The ^{13}C-NMR spectrum *(Exper. Part)* of (+)-**1** shows 1 –COO–, 1 –O–CH–, 10 CH, 9 CH_2, and 1 CH_3 group. Methylation (→**3**) and silylation (→**4**) reveal that the original oxygenated functions are –COOH and HO–CH–. These data and the molecular ion at *m/z* 346 give the composition $C_{22}H_{34}O_3$ for (+)-**1**; on account also of the presence of only 1 Me group and of disubstitution at all olefinic bonds, the acid must be linear. The fragments C(2) to C(7), C(8) to C(13), C(14) to C(16), C(17) to C(19), and C(20) to C(22) of (+)-**1** are established by COSY experiments [7]; adaptation to long-range coupling [7] further reveals the fragments C(6) to C(9), and C(13) to C(22). Finally, a typical δ(H) of 4.14 ppm for H–C(10) in the ^{1}H-NMR spectrum in C_6D_6[2]) completes the gross structure.

The configuration in the fragment C(11) to C(14) of (+)-**1** is suggested by a typical UV spectrum [4c] and confirmed by the relatively large H,H coupling constants J(11,12) = 15.0 and J(13,14) = 11.0 Hz and by a +5% differential NOE between CH(12) and CH_2(15). The (*Z*)-configuration for the C(7)=C(8) and C(19)=C(20) bonds is based on the typical H,H coupling constants J(7,8) = 10.5 and J(19,20) = 10.7 Hz and on a 2% NOE between CH_2(18) and CH_2(21). Finally, the (*Z*)-configuration at the C(16)=C(17) bond, where the ^{1}H-NMR spectrum is of no aid because of superimpositions of signals, rests on the typically shielded resonance for C(15) and C(18) [8] [9] (δ = 26.10 and 25.56 ppm, resp., in $CDCl_3$).

The ethyl ester (+)-**2** shows NMR spectra superimposable with those for (+)-**1**, except for additional resonances due to the ester moiety (see *Exper. Part*).

Scheme



a) NaOMe (0.01M), MeOH, r.t., 10 min. *b)* CH_2N_2, Et_2O. *c)* $(PhCO)_2O$, pyridine, r.t., 10 h. *d)* 1. O_3, CH_2Cl_2, –78°; 2. 12% H_2O_2, AcOH, 60°, 30 min; 3. CH_2N_2, Et_2O. *e)* (–)-(*S*)-1-phenylethyl isocyanate, toluene, 90°, 13 h.

The (10*R*)-configuration of (+)-**1** and (+)-**2** is established by their correlation with (+)-(*R*)-hydroxysuccinic acid ((+)-**7**; see *Scheme*). Thus, (+)-**1** or (+)-**2** is first transformed to the methyl ester (+)-**3**, which is then 10-*O*-benzoylated (→(+)-**5**), ozonized, and methylated to (+)-**6**. The latter proves to be identical to the benzoate obtained from commercial (+)-**7** *via* (+)-**8**, according to ^{1}H-NMR spectra in the presence of the chiral shift reagent Yb(tfc)$_3$[3]). Alternatively, (+)-**3** is transformed into urethane **9** and then

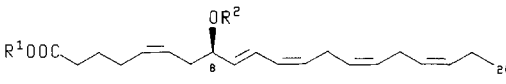
[2]) The ^{1}H-NMR spectrum of (+)-**1** was also run in $CDCl_3$ to complement information for overcrowded zones.

[3]) Comparison with the spectra of the benzoate of (±)-**8** shows that, at [Yb(tfc)$_3$]/[substrate] ≈ 0.2, the lanthanide-induced shift in $CDCl_3$ for MeOOC(4) is 138 and 132 Hz in the case of (2*R*)- and (2*S*)-configuration, respectively.

ozonized and methylated to (−)-**10** whose HPLC and ^{1}H-NMR data prove identical to those of the product prepared from commercial (+)-**7**[4]).

The presence of a single, optically active hydroxy compound rules out any autooxidation process from fatty acid precursors (which are known to lead to a mixture of products [10]) for the formation of leiopathic acid. The presence of the ethyl ester (+)-**2** in the ethanolic extracts from *Leiopathes* sp. suggests that at least part of leiopathic acid must be bound by easily solvolyzable bonds to other compounds in the animal, possibly to cellular walls.

3. Icosapentaenoic Acid and Ester. – Another fatty acid, (+)-**11**, which is more polar than (+)-**1**, and its ethyl ester (+)-**12**, which is more polar than (+)-**2**, are isolated from the *Leiopathes* sp. and prove to be of the C_{20} type.



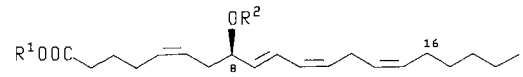
(+)-**11** $R^1 = R^2 = H$
(+)-**12** R^1 = Et, $R^2 = H$
(+)-**13** R^1 = Me, $R^2 = H$
14 R^1 = Me, $R^2 = Me_3Si$
15 R^1 = Me, R^2 = (*S*)-MeCH(Ph)NHCO

The C(5) to C(20) portion of the NMR spectra of (+)-**11**[5]) is nearly superimposable to the corresponding C(7) to C(22) portion of (+)-**1**, but (+)-**11** lacks the signals for 2 CH_2 groups. This is in accordance with MS data *(Exper. Part)* and leads to structure (+)-**11** which is further supported by COSY experiments and also by its esterification (→(+)-**13**) followed by silylation (→**14**). The structure of (+)-**12** is established along similar lines as for (+)-**2** above.

The (8*R*)-configuration of (+)-**11** and (+)-**12** is established as for (+)-**1** and (+)-**2** by correlation to (+)-**7** *via* (−)-**10** and (+)-**8** (see *Scheme*).

The racemate (±)-**13** is reported to be a product of the peroxidation of methyl 5,8,11,14,17-icosapentaenoate, after $NaBH_4$ reduction [11]; its structure relies on established reaction mechanisms, and no direct structural proof is given.

4. Icosatetraenoic Acid and Ester. – Another C_{20} fatty acid, (+)-**16**, and its ethyl ester (+)-**17** are isolated from the *Leiopathes* sp.



(+)-**16** $R^1 = R^2 = H$
(+)-**17** R^1 = Et, $R^2 = H$
(+)-**18** R^1 = Me, $R^2 = H$
19 R^1 = Me, $R^2 = Me_3Si$

The C(1) to C(15) portion of the NMR spectra of (+)-**16** is practically superimposable to the corresponding portion of (+)-**11**; the only difference is that a *cis* CH=CH group of (+)-**11** is replaced by 2 CH_2 groups in (+)-**16**[6]). This is in accordance with MS data *(Exper. Part)*, leading to structure (+)-**16**. As a further structural confirmation, esterification of (+)-**16** with diazomethane leads to (+)-**18** which is silylated (→**19**); as (−)-**18** is known [12], this also proves the absolute configuration of (+)-**18**. The structure of ethyl ester (+)-**17** is deduced along similar lines as for (+)-**2** and (+)-**12**.

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[4]) The (2*S*)-diastereoisomer of (−)-**10** has a *ca.* 7% smaller t_R in the HPLC (hexane/AcOEt 3:1) than (−)-**10** (2*R*), and in its ^{1}H-NMR spectrum ($CDCl_3$), MeOOC(1) and MeOOC(4) appear at 3.67 and 3.77 ppm, respectively, as compared to 3.71 and 3.72 ppm for (−)-**10** (2*R*).

[5]) The NMR resonances of (+)-**11** are of increasing broadness from CH_2(4) to C(1), possibly owing to a slow equilibrium between the hydroxy acid and lactonic forms. In agreement, for the corresponding esters (+)-**12** and (+)-**13** all resonances are normally sharp.

[6]) As in the case of (+)-**11** the NMR resonances of (+)-**16** are of increasing broadness from CH(8) to C(1).

Compound **16** has been described as resulting from the transformation of arachidonic acid by rat hepatic microsomal cytochrome P-450 [13]. Chromatographic comparison with diastereoisomers of known configuration has established its (8*R*)-configuration, though neither chiroptical nor spectral data have been reported [4b].

The (8*R*)-hydroperoxy analogue of (+)-**16** has been obtained from the C(8) lipoxygenase of arachidonic acid with homogenates of the gorgonian *Pseudoplexaura porosa* [4a].

The antipatharian was obtained thanks to the generous interest of Dr. *P. M. Arnaud,* chief scientist of the MD-50 cruise, to whom we are most indebted, and to the financial and logistic support of Terres Australes et Antarctiques Françaises. We thank also Mr. *N. Demattè* for skilled aid in the separation of the product, Dr. *M. Grasshoff* for the identification of the coral, Mr. *L. Zuppiroli,* Facoltà di Chimica Industriale, Università di Bologna, for *VG70-70* mass spectrometric measurements, and, for support of the work in Trento, the *C. N. R.* and the *M. P. I.* (Progetti 40%), Roma.

Experimental Part

1. *General.* TLC: *Merck silica gel 60 PF_{254}* plates. HPLC: *Merck LiChrosorb Si-60* (7 μm); reverse-phase HPLC: *Merck LiChrosorb RP-18* (7 μm); 25 × 1 cm columns, flux 5 ml/min. Flash chromatography (FC): *Merck LiChroprep Si-60* (15–25 μm) or, only for the initial phase of isolation of natural products, *Merck* silica gel *60* (70–230 μm). Reverse-phase FC: *Merck RP-18 LiChroprep* (40–65 μm). All evaporations were carried out at reduced pressure at r.t. Reaction yields are calculated on reacted compounds. Polarimetric data: *JASCO-DIP-181* digital polarimeter. UV spectra: *Perkin-Elmer Lambda-3* spectrophotometer; λ_{max} in nm, ε in $dm^3 \cdot mol^{-1} \cdot cm^{-1}$. ^{1}H-NMR and ^{13}C-NMR spectra (at 21°, unless otherwise stated): *Varian XL300* (300 or 75.43 MHz, resp.); δ (ppm) relative to internal Me_4Si (= 0 ppm) and J in Hz; coupling constants are derived from homonuclear decoupling, differential NOE, preirradiation of 8 sec (irradiated proton(s)→% increment (relaxed proton(s)); ^{13}C multiplicities by DEPT [14] techniques; chemical-shift assignments are supported by ^{13}C,^{1}H-NMR shift correlation experiments (HETCOR) [15], comparison with data for related compounds [8], and calculations of chemical shifts [8] [16]. Low-resolution MS: home-built quadrupole mass spectrometer based on the *ELFS-4-162-8 Extranuclear* quadrupole [17] or (for (+)-**1**) *VG 70-70* mass spectrometer.

2. *Collection and Isolations. Leiopathes* sp. was collected by beam trawl during the cruise MD-50 Jasus of M/S 'Marion-Dufresne' West of Saint Paul Island, 38° 43.53′ to 38° 43.46′ S – 77° 26.50′ to 77° 26.04′ E, Sta. 27, CP129, depth 290 m, and immediately immersed in 95% EtOH with the horny skeleton to fill two glass jars of 2 l each. Identification by Dr. *M. Grasshoff,* Forschungsinstitut Senckenberg, Frankfurt am Main.

Thorough extraction of the colonies with EtOH and then with acetone was followed by evaporation and extraction of the aqueous residue with EtOAc. Evaporation led to 22.8 g of a dark residue, the half of which was subjected to FC on silica gel, gradient elution with petroleum ether/Et_2O/EtOAc/MeOH. Polar fractions, eluted with EtOAc/MeOH, gave 1.1 g of residue *A*. Fractions eluted with petroleum ether/Et_2O yielded 3.2 g of residue *B*. Residue *A* was subjected to *RP18* FC; with MeOH/H_2O 9:1 or MeOH/H_2O 8:2, we obtained residues A^1 (0.222 g) and A^2 (0.084 g), resp. Residue A^1 was subjected to reverse-phase HPLC with MeOH/H_2O/AcOH 80:20:0.25, and the eluates were partially evaporated and subjected to *RP18* FC to remove AcOH; evaporations gave (+)-**1** (0.046 g, 0.4%) at t_R 26 min and (+)-**16** (0.011 g, 0.1%) at t_R 23.5. Residue A^2 was subjected to the same procedure, except for using MeOH/H_2O/AcOH 85:15:0.25, to give (+)-**11** (0.04 g, 0.3%) at t_R 7.7 min. Residue *B* was subjected to reverse-phase FC, gradient MeOH/H_2O; the residue of the eluate with MeOH/H_2O 9:1 was subjected to HPLC with hexane/AcOEt 17:3 to give (+)-**2** (11 mg, 0.09%) at t_R 10.8 min, (+)-**17** (3 mg, 0.03%) at t_R 12.9 min, and (+)-**12** (11 mg, 0.1%) at t_R 14.1.

3. *Leiopathic Acid (= (+)-(10R,7Z,11E,13Z,16Z,19Z)-10-Hydroxy-7,11,13,16,19-docosapentaenoic Acid* ((+)-**1**)). $[\alpha]^{20}$: +3.1 (589), +3.8 (577), +5.0 (546), −350.7 (435; c = 1.85, $CHCl_3$). UV (95% EtOH): 236.3 (24800). ^{1}H-NMR ($CDCl_3$): 2.28 (*m*, 2 H–C(2), 2 H–C(9)); 1.58 (*tt*, J = 7.0, 7.0, 2 H–C(3)); 1.32 (*m*, 2 H–C(4), 2 H–C(5)); 2.02 (*m*, 2 H–C(6), 2 H–C(21)); 5.50 (br. *dt*, J = 10.5, 6.9, H–C(7); irradiation at 2.02→br. *d*, J = 10.5; irradiation at 2.28→*dt*, J = 10.5, 6.9); 5.35 (*m*, H–C(8), H–C(14), H–C(16), H–C(17), H–C(20)); 4.17 (br. *dt*, J = 6.3, 6.3, H–C(10)); 5.78 (br. *dd*, J = 15.0, 6.3, H–C(11)); 6.50 (br. *dd*, J = 15.0, 11.0, H–C(12)); 5.95 (br. *dd*, J = 11.0, 11.0, H–C(13)); 2.91 (br. *dd*, J = 6.5, 6.5, 2 H–C(15)); 2.76 (br. *dd*, J = 7.0, 7.0, 2 H–C(18)); 5.26 (*dtt*, J = 10.7, 7.0, 1.2, H–C(19); irradiation at 2.02→*dt*, J = 10.7, 7.0; irradiation at 2.76→*dt*, J = 10.7, 1.2); 0.96 (*t*,

J = 7.6, 3 H–C(22)). ^{1}H-NMR (C_6D_6): 2.11 (*t*, J = 7.2, 2 H–C(2)); 1.48 (*tt*, J = 7.2, 7.2, 2 H–C(3)); 1.16 (*m*, 2 H–C(4)); 1.18 (*m*, 2 H–C(5)); 1.94 (*m*, 2 H–C(6)); 2.31 (*m*, 2 H–C(9); irradiation at 5.44→*AB* of *ABX*); 4.14 (br. *dt*, J = 6.2, 6.2, H–C(10)); 5.69 (*dd*, J = 15.0, 6.2, H–C(11)); 6.67 (br. *dd*, J = 15.0, 11.0, H–C(12)); 6.06 (br. *dd*, J = 11.0, 11.0, H–C(13)); 2.96 (br. *dd*, J = 6.3, 6.3, 2 H–C(15)); 2.80 (*m*, 2 H–C(18)); 2.01 (*m*, 2 H–C(21)); 0.92 (*t*, J = 7.5, 3 H–C(22)); the remaining olefinic protons at 5.47 (H–C(7)), 5.46 (H–C(8)), 5.43 (H–C(14)), 5.42 (H–C(16)), 5.45 (H–C(17)), 5.40 (H–C(19)), and 5.42 (H–C(20)) were assigned from slices along the f_2 axis in the tridimensional 2-quantum filtered COSY plot [18]; positive NOE: H–C(10)→5 (2 H–C(9)), 2 (H–C(12)); H–C(11)→2 (2 H–C(9)), 4 (H–C(13)); H–C(12)→5 (2 H–C(15)), 4 (H–C(10)); 2 H–C(18)→2 (2 H–C(21)). ^{13}C-NMR ($CDCl_3$ values for C_6D_6 in brackets): 178.85 [179.69] (*s*, C(1)); 33.86 [34.24] (*t*, C(2)); 24.54 [24.86] (*t*, C(3)); 28.65 [28.85] (*t*, C(4)); 29.13 [29.46] (*t*, C(5)); 27.18 [27.48] (*t*, C(6)); 133.16 [132.78] (*d*, C(7)); 124.61 [125.35] (*d*, C(8)); 35.39 [35.86] (*t*, C(9)); 72.13 [72.31] (*d*, C(10)); 135.57 [136.54] (*d*, C(11)); 125.57 [125.49] (*d*, C(12)); 127.94 [128.73] (*d*, C(13)); 130.43 [130.18] (*d*, C(14)); 26.10 [26.47] (*t*, C(15)); 127.38 [127.84] (*d*, C(16)); 128.95 [129.18] (*d*, C(17)); 25.56 [25.92] (*t*, C(18)); 126.93 [127.41] (*d*, C(19)); 132.11 [132.18] (*d*, C(20)); 20.56 [20.92] (*t*, C(21)); 14.23 [14.50] (*q*, C(22)). MS: 346 (4, M^+), 328 (8, $M^+ - 18$), 302 (3, $M^+ - 44$), 237 (15), 191 (60), 173 (37), 91 (64), 83 (100).

4. *Transformation of (+)-***1** *into (+)-***6**. A soln. of (+)-**1** (0.013 g, 0.038 mmol) in 1 ml of Et_2O was added to ethereal CH_2N_2 in excess to give (+)-**3** in quantitative yield on evaporation. The latter in 0.2 ml of pyridine was treated with 2 mol-equiv. of $(PhCO)_2O$ at r.t. for 10 h. Evaporation and TLC (hexane/Et_2O 8:2) gave, at R_f 0.5, (+)-**5** in 75% yield. The latter in 1 ml of CH_2Cl_2 at −78° was treated with excess O_3. The mixture was flushed with N_2 and evaporated, and 12% H_2O_2 soln. (0.3 ml) and AcOH (2 drops) were added to the residue and heated at 60° for 30 min. Evaporation, treatment with excess ethereal CH_2N_2, and TLC with hexane/Et_2O/AcOH 6:4:0.1 led to (+)-**6**, R_f 0.4 (0.002 g, 30%).

*(+)-(10*R*,7*Z*,11*E*,13*Z*,16*Z*,19*Z*)-Methyl 10-Hydroxy-7,11,13,16,19-docosapentaenoate* ((+)-**3**). $[\alpha]^{20}$: +15.5 (435), +26.6 (365; c = 0.34, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 3.66 (*s*, MeO); 2.30 (*t*, J = 7.5, 2 H–C(2)); 1.62 (*tt*, J = 7.5, 7.5, 2 H–C(3)); 1.35 (*m*, 2 H–C(4), 2 H–C(5)); 2.07 (*m*, 2 H–C(6), 2 H–C(21)); 5.55 (*dtt*, J = 10.8, 7.1, 1.4, H–C(7)); 5.35–5.45 (*m*, H–C(8), H–C(14), H–C(16), H–C(17), H–C(20)); 2.33 (*m*, 2 H–C(9)); 4.22 (*m*, H–C(10)); 1.70 (br. *d*, J = 3.8, OH); 5.72 (*dd*, J = 15.2, 6.5, H–C(11)); 6.55 (*dddd*, J = 15.2, 11.0, 1.2, 1.2, H–C(12)); 6.00 (br. *dd*, J = 11.0, 11.0, H–C(13)); 2.97 (br. *dd*, J = 6.5, 6.5, 2 H–C(15)); 2.81 (br. *dd*, J = 6.7, 6.7, 2 H–C(18)); 5.32 (*dtt*, J = 10.6, 7.1, 1.4, H–C(19)); 0.97 (*t*, J = 7.4, 3 H–C(22)). ^{13}C-NMR ($CDCl_3$): 51.41 (*q*, MeO); 174.16 (*s*, C(1)); 34.02 (*t*, C(2)); 24.81 (*t*, C(3)); 28.75 (*t*, C(4)); 29.18 (*t*, C(5)); 27.22 (*t*, C(6)); 133.23 (*d*, C(7)); 124.58 (*d*, C(8)); 35.43 (*t*, C(9)); 72.08 (*d*, C(10)); 135.67 (*d*, C(11)); 125.51 (*d*, C(12)); 127.96 (*d*, C(13)); 130.40 (*d*, C(14)); 26.10 (*t*, C(15)); 127.39 (*d*, C(16)); 128.95 (*d*, C(17)); 25.57 (*t*, C(18)); 126.93 (*d*, C(19)); 132.11 (*d*, C(20)); 20.56 (*t*, C(21)); 14.23 (*q*, C(22)). MS: 342 (15, $M^+ - 18$), 91 (100).

*(+)-(10*R*,7*Z*,11*E*,13*Z*,16*Z*,19*Z*)-Methyl 10-(Benzoyloxy)-7,11,13,16,19-docosapentaenoate* ((+)-**5**). ^{1}H-NMR ($CDCl_3$): 3.67 (*s*, MeO); 2.28 (*t*, J = 7.5, 2 H–C(2)); 1.58 (*tt*, J = 7.5, 7.5, 2 H–C(3)); 1.32 (*m*, 2 H–C(4), 2 H–C(5)); 2.05 (*m*, 2 H–C(6), 2 H–C(21)); 5.25–5.56 (*m*, H–C(7), H–C(8), H–C(14), H–C(16), H–C(17), H–C(20)); 2.54 (*m*, 2 H–C(9)); 5.59 (br. *dt*, J = 7.0, 7.0, H–C(10)); 8.05, 7.55, 7.44 (arom. H_o, H_p, H_m); 5.75 (*dd*, J = 15.2, 7.0, H–C(11)); 6.64 (*dddd*, J = 15.2, 11.1, 1.3, 1.3, H–C(12)); 5.99 (br. *dd*, J = 11.1, 11.1, H–C(13)); 2.95 (br. *dd*, J = 6.5, 6.5, 2 H–C(15)); 2.79 (br. *dd*, J = 6.4, 6.4, 2 H–C(18)); 5.30 (*dtt*, J = 10.6, 6.4, 1.3, H–C(19)); 0.97 (*t*, J = 7.5, 3 H–C(22)).

*(+)-(*R*)-Dimethyl 2-(Benzoyloxy)butanedioate* ((+)-**6**). ^{1}H-NMR ($CDCl_3$): 3.79 (*s*, MeO–C(1)); 5.73 (*X* of *ABX*, $J(AX)$ = 6.8, $J(BX)$ = 5.5, H–C(2)); 8.05, 7.59, 7.45 (arom. H_o, H_p, H_m); 3.046, 3.042 (*AB* of *ABX*, $J(AB)$ = 13.5, 2 H–C(3)); 3.73 (*s*, MeO–C(4)).

5. *Transformation of (+)-***3** *into (−)-***10**. A soln. of (+)-**3** (0.006 g, 0.017 mmol) and 2 mol-equiv. of (−)-(*S*)-1-phenylethyl isocyanate in 1 ml of toluene was heated at 90° for 13 h. Evaporation and TLC (hexane/Et_2O 1:1) led to **9** in 45% yield, R_f 0.7. Following the methodology for (+)-**5**→(+)-**6** *(Exper. 4)*, **9** was transformed into (−)-**10** (0.0007 g, 28%). TLC (Et_2O/hexane 6:4): R_f 0.3.

*(10*R*,7*Z*,11*E*,13*Z*,16*Z*,19*Z*)-Methyl 10-[(*S*)-(1-Phenylethyl)carbamoyloxy]-7,11,13,16,19-docosapentaenoate* (**9**). ^{1}H-NMR ($CDCl_3$): 3.67 (*s*, MeO); 2.31 (*t*, J = 7.4, 2 H–C(2)); 1.56 (*tt*, J = 7.4, 7.4, 2 H–C(3)); 1.30 (*m*, 2 H–C(4), 2 H–C(5)); 2.05 (*m*, 2 H–C(6), 2 H–C(21)); 5.25–5.55 (*m*, H–C(7), H–C(8), H–C(14), H–C(16), H–C(17), H–C(19), H–C(20), NH); 2.39 (*m*, 2 H–C(9)); 5.22 (*dt*, J = 6.8, 6.8, H–C(10)); 4.90 (*dq*, J = 7.1, 7.1, CH_3CH); 1.49 (*d*, J = 7.1, CH_3CH); 7.18, 7.47 (superimposed arom. H); 5.61 (*dd*, J = 15.0, 6.8, H–C(11)); 6.50 (br. *dd*, J = 15.0, 11.0, H–C(12)); 5.96 (br. *dd*, J = 11.0, 11.0, H–C(13)); 2.93 (br. *dd*, J = 6.5, 6.5, 2 H–C(15)); 2.80 (br. *dd*, J = 6.4, 6.4, 2 H–C(18)); 0.97 (*t*, J = 7.5, 3 H–C(22)).

(−)-(2R)-Dimethyl [(S)-(1-Phenylethyl)carbamoyloxy]butanedioate ((−)-**10**). ^{1}H-NMR ($CDCl_3$): 3.71, 3.72 (2*s*, 2 MeO); 5.42 (*X* of *ABX*, *J*(*AX*) = 6.5, *J*(*BX*) = 4.9, H–C(2)); 5.25 (br. *d*, *J* = 6.9, NH); 4.83 (*dq*, *J* = 6.9, 6.9, CH_3CH); 1.48 (*d*, *J* = 6.9, CH_3CH); 7.20, 7.40 (superimposed arom. H); 2.892, 2.889 (*AB* of *ABX*, *J*(*AB*) = 14.0, 2 H–C(3)).

6. *(+)-(10R,7Z,11E,13Z,16Z,19Z)-Ethyl 10-Hydroxy-7,11,13,16,19-docosapentaenoate* ((+)-**2**). $[\alpha]^{20}$: +5.3 (589), +6.0 (577), +11.7 (435), +24.7 (365; *c* = 0.53, $CHCl_3$). UV (95% EtOH): 236.5 (26500). ^{1}H-NMR (C_6D_6): 3.97 (*q*, *J* = 7.1, CH_3–CH_2O); 0.97 (*t*, *J* = 7.1, CH_3CH_2O); 2.12 (*t*, *J* = 7.3, 2 H–C(2)); 1.54 (*tt*, *J* = 7.3, 7.3, 2 H–C(3)); 1.18, 1.21 (2*m*, 2 H–C(4), 2 H–C(5)); 1.94, 1.99 (2*m*, 2 H–C(6), 2 H–C(21)); 5.35–5.50 (*m* H–C(7), H–C(8), H–C(14), H–C(16), H–C(17), H–C(19), H–C(20)); 2.26 (*m*, 2 H–C(9)); 4.06 (*dt*, *J* = 6.4, 6.4, H–C(10)); 5.65 (*dd*, *J* = 15.1, 6.4, H–C(11)); 6.88 (br. *dd*, *J* = 15.1, 11.0, H–C(12)); 6.06 (br. *dd*, *J* = 11.0, 11.0, H–C(13)); 2.96 (br. *dd*, *J* = 6.4, 6.4, 2 H–C(15)); 2.80 (*m*, 2 H–C(18)); 0.91 (*t*, *J* = 7.5, 3 H–C(22)). ^{13}C-NMR (C_6D_6): 60.00 (*t*, CH_3CH_2O); 14.33 (*q*, CH_3CH_2O); 173.00 (*s*, C(1)); 34.34 (*t*, C(2)); 25.15 (*t*, C(3)); 28.96 (*t*, C(4)); 29.52 (*t*, C(5)); 27.50 (*t*, C(6)); 132.85 (*d*, C(7)); 125.41 (*d*, C(8)); 35.97 (*t*, C(9)); 72.00 (*d*, C(10)); 136.87 (*d*, C(11)); 125.19 (*d*, C(12)); 128.77 (*d*, C(13)); 129.99 (*d*, C(14)); 26.46 (*t*, C(15)); 127.85 (*d*, C(16)); 129.15 (*d*, C(17)); 25.92 (*t*, C(18)); 127.40 (*d*, C(19)); 132.16 (*d*, C(20)); 20.91 (*t*, C(21)); 14.47 (*q*, C(22)). MS: 356 (11, M^{+} − 18), 91 (100).

7. *Transformation of (+)-2 into (+)-3*. To 1 ml of 0.01M NaOMe in MeOH was added (+)-**2** (0.011 g, 0.029 mmol). After 10 min, the mixture was partially evaporated and subjected to HPLC (hexane/AcOEt 4:1) to give (+)-**3** (7.5 mg, 84%) at t_R 7.8 min, besides 1.6 mg of unreacted (+)-**2**. Spectral and chiroptical data: identical to those above.

8. *(+)-(8R,5Z,9E,11Z,14Z,17Z)-8-Hydroxy-5,9,11,14,17-icosapentaenoic Acid* ((+)-**11**). UV (95% EtOH): 236.3 (21000). $[\alpha]^{20}$: +3.0 (589), +3.3 (577), +4.4 (546), −379.8 (435; *c* = 0.43, $CHCl_3$). ^{1}H-NMR (C_6D_6): 2.10 (br. *t*, *J* = 7.0, 2 H–C(2)); 1.53 (br. *tt*, *J* = 7.0, 7.0, 2 H–C(3)); 1.92 (br. *td*, *J* = 7.0, 7.0, 2 H–C(4)); 5.30–5.50 (*m*, H–C(5), H–C(6), H–C(12), H–C(14), H–C(15), H–C(17), H–C(18)); 2.24 (*m*, 2 H–C(7)); 4.08 (br. *dt*, *J* = 6.2, 6.2, H–C(8)); 5.65 (br. *dd*, *J* = 15.0, 6.1, H–C(9)); 6.68 (br. *dd*, *J* = 15.0, 11.0, H–C(10)); 6.07 (br. *dd*, *J* = 11.0, 11.0, H–C(11)); 2.97 (br. *dd*, *J* = 6.3, 6.3, 2 H–C(13)); 2.81 (*m*, 2 H–C(16)); 2.01 (*m*, 2 H–C(19)); 0.93 (*t*, *J* = 7.5, 3 H–C(20)). ^{13}C-NMR (C_6D_6): 179.34 (br. *s*, C(1)); 33.44 (br. *t*, C(2)); 24.76 (br. *t*, C(3)); 26.81 (br. *t*, C(4)); 131.61 (*d*, C(5)); 126.41 (*d*, C(6)); 35.80 (*t*, C(7)); 72.17 (*d*, C(8)); 136.59 (*d*, C(9)); 125.46 (*d*, C(10)); 128.72 (*d*, C(11)); 130.16 (*d*, C(12)); 26.48 (*t*, C(13)); 127.85 (*d*, C(14)); 129.19 (*d*, C(15)); 25.93 (*t*, C(16)); 127.41 (*d*, C(17)); 132.19 (*d*, C(18)); 20.92 (*t*, C(19)); 14.48 (*q*, C(20)). MS: 300 (2, M^{+} − 18), 91 (100).

9. *Transformation of (+)-11 into (−)-10*. Following the procedures of *Exper. 4*, (+)-**11** (0.015 g, 0.047 mmol) gave quantitatively (+)-**13** which was transformed into (−)-**10** in 25% overall yield. Data for (−)-**10**: coincident with those above.

(+)-(8R,5Z,9E,11Z,14Z,17Z)-Methyl 8-Hydroxy-5,9,11,14,17-icosapentaenoate ((+)-**13**). $[\alpha]^{20}$: +20.9 (435), +39.0 (365; *c* = 0.11, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 3.67 (*s*, MeO); 2.32 (*t*, *J* = 7.5, 2 H–C(2)); 1.71 (*tt*, *J* = 7.5, 7.5, 2 H–C(3)); 2.10 (*m*, 2 H–C(4), 2 H–C(19)); 5.55 (*dtt*, *J* = 11.8, 7.0, 1.4, H–C(5)); 5.35–5.53 (*m*, H–C(6), H–C(12), H–C(14), H–C(15), H–C(18)); 2.33 (*m*, 2 H–C(7)); 4.23 (*dt*, *J* = 6.5, 6.5, H–C(8)); 1.75 (br. *s*, OH); 5.72 (*dd*, *J* = 15.2, 6.5, H–C(9)); 6.56 (*dddd*, *J* = 15.2, 11.0, 1.3, 1.3, H–C(10)); 6.02 (br. *dd*, *J* = 11.0, 11.0, H–C(11)); 2.97 (br. *dd*, *J* = 6.5, 6.5, 2 H–C(13)); 2.82 (br. *dd*, *J* = 6.5, 6.5, 2 H–C(16)); 5.33 (*dtt*, *J* = 10.5, 7.0, 1.4, H–C(17)); 0.97 (*t*, *J* = 7.4, 3 H–C(20)).

10. *(+)-(8R,5Z,9E,11Z,14Z,17Z)-Ethyl 8-Hydroxy-5,9,11,14,17-icosapentaenoate* ((+)-**12**). $[\alpha]^{20}$: +3.7 (589), +3.9 (577), +5.2 (546), +10.1 (435), +19.9 (365; *c* = 0.33, $CHCl_3$). UV (95% EtOH): 236.5 (22200). ^{1}H-NMR (C_6D_6): 3.94 (*q*, *J* = 7.0, CH_3CH_2O); 0.96 (*t*, *J* = 7.0, CH_3CH_2O); 2.09 (*t*, *J* = 7.2, 2 H–C(2)); 1.58 (*tt*, *J* = 7.2, 7.2, 2 H–C(3)); 1.94 (*dt*, *J* = 7.2, 7.2, 2 H–C(4)); 5.30–5.50 (*m*, H–C(5), H–C(6), H–C(12), H–C(14), H–C(15), H–C(17), H–C(18)); 2.21 (*m*, 2 H–C(7)); 4.04 (*dt*, *J* = 6.5, 6.5, H–C(8)); 5.63 (br. *dd*, *J* = 15.0, 6.5, H–C(9)); 6.67 (br. *dd*, *J* = 15.0, 11.1, H–C(10)); 6.06 (br. *dd*, *J* = 11.1, 11.1, H–C(11)); 2.96 (br. *dd*, *J* = 6.6, 6.6, 2 H–C(13)); 2.80 (*m*, 2 H–C(16)); 2.00 (*dq*, *J* = 7.3, 7.3, 2 H–C(19)); 0.90 (*t*, *J* = 7.3, 3 H–C(20)). ^{13}C-NMR (C_6D_6): 60.07 (*t*, CH_3CH_2O); 14.30 (*q*, CH_3CH_2O); 172.98 (*s*, C(1)); 33.60 (*t*, C(2)); 25.06 (*t*, C(3)); 26.93 (*t*, C(4)); 131.75 (*d*, C(5)); 126.41 (*d*, C(6)); 35.89 (*t*, C(7)); 71.91 (*d*, C(8)); 136.86 (*d*, C(9)); 125.17 (*d*, C(10)); 128.76 (*d*, C(11)); 129.97 (*d*, C(12)); 26.46 (*t*, C(13)); 127.86 (*d*, C(14)); 129.15 (*d*, C(15)); 25.92 (*t*, C(16)); 127.40 (*d*, C(17)); 132.16 (*d*, C(18)); 20.92 (*t*, C(19)); 14.47 (*q*, C(20)). MS: 328 (7, M^{+} − 18), 91 (100).

11. *Transformation of (+)-12 into (+)-13*. Following the procedure of *Exper. 7*, (+)-**12** was transformed into (+)-**13** in 90% yield.

12. *(+)-(8R,5Z,9E,11Z,14Z)-8-Hydroxy-5,9,11,14-icosatetraenoic Acid* ((+)-**16**). $[\alpha]^{20}$: +4.0 (589), +4.6 (577), +5.9 (546), −113.6 (435), −376.9 (365; *c* = 0.48, $CHCl_3$). UV (95% EtOH): 236.5 (21150). ^{1}H-NMR (C_6D_6,

30°): 1.9–2.3 (br. *m*, 2 H–C(2), 2 H–C(4), 2 H–C(7)); 1.55 (br. *m*, 2 H–C(3)); 5.30–5.55 (*m*, H–C(5), H–C(6), H–C(12), H–C(14), H–C(15)); 4.09 (br. *m*, H–C(8)); 5.66 (br. *dd*, *J* = 15.0, 6.0, H–C(9)); 6.69 (br. *dd*, *J* = 15.0, 11.0, H–C(10)); 6.08 (br. *dd*, *J* = 11.0, 11.0, H–C(11)); 2.98 (br. *dd*, *J* = 6.1, 6.1, 2 H–C(13)); 2.05 (br. *dt*, *J* = 6.3, 6.3, 2 H–C(16)); 1.3–1.4 (*m*, 2 H–C(17), 2 H–C(18)); 1.26 (*m*, 2 H–C(19)); 0.89 (*t*, *J* = 7.5, 3 H–C(20)). ^{13}C-NMR (C_6D_6): 179.16 (br. *s*, C(1)); 33.40 (br. *t*, C(2)); 24.80 (br. *t*, C(3)); 26.82 (br. *t*, C(4)); 131.62 (br. *d*, C(5)); 126.45 (br. *d*, C(6)); 35.82 (br. *t*, C(7)); 72.13 (br. *d*, C(8)); 136.53 (*d*, C(9)); 125.52 (*d*, C(10)); 128.62 (*d*, C(11)); 130.49 (*d*, C(12)); 26.55 (*t*, C(13)); 127.34 (*d*, C(14)); 130.99 (*d*, C(15)); 27.59 (*t*, C(16)); 29.71 (*t*, C(17)); 31.83 (*t*, C(18)); 22.98 (*t*, C(19)); 14.33 (*q*, C(20)). MS: 302 (7, $M^+ - 18$), 91 (100).

13. *Methylation of (+)-***16**. Following the procedure of *Exper. 4*, (+)-**16** (0.005 g, 0.016 mmol) gave quantitatively (+)-**18**. ^{1}H-NMR data and absolute value of [α]: matching those given for the enantiomer [12].

14. *(+)-(8*R*,5*Z*,9*E*,11*Z*,14*Z*)-Ethyl 8-Hydroxy-5,9,11,14-icosatetraenoate* ((+)-**17**). $[\alpha]_{20}$: +4.5 (589), +8.9 (546), +15.9 (435), +27.7 (365; *c* = 0.32, $CHCl_3$). UV (95% EtOH): 236.5 (18700). ^{1}H-NMR (C_6D_6): 3.96 (*q*, *J* = 7.0, CH_3CH_2O); 0.97 (*t*, *J* = 7.0, CH_3CH_2O); 2.10 (*t*, *J* = 7.4, 2 H–C(2)); 1.59 (*tt*, *J* = 7.4, 7.4, 2 H–C(3)); 1.94 (*dt*, *J* = 7.4, 7.4, 2 H–C(4)); 5.30–5.50 (*m*, H–C(5), H–C(6), H–C(12), H–C(14), H–C(15)); 2.22 (*m*, 2 H–C(7)); 4.03 (br. *dt*, *J* = 6.3, 6.3, H–C(8)); 5.63 (br. *dd*, *J* = 15.1, 6.3, H–C(9)); 6.69 (br. *dd*, *J* = 15.1, 11.0, H–C(10)); 6.07 (br. *dd*, *J* = 11.0, 11.0, H–C(11)); 2.97 (br. *dd*, *J* = 6.3, 6.3, 2 H–C(13)); 2.01 (*m*, 2 H–C(16)); 1.22–1.35 (*m*, 2 H–C(17), 2 H–C(18), 2 H–C(19)); 0.88 (*t*, *J* = 7.3, 3 H–C(20)). ^{13}C-NMR (C_6D_6): 60.07 (*t*, CH_3CH_2O); 14.31 (*q*, CH_3CH_2O); 172.99 (*s*, C(1)); 33.61 (*t*, C(2)); 25.06 (*t*, C(3)); 26.93 (*t*, C(4)); 131.75 (*d*, C(5)); 126.43 (*d*, C(6)); 35.89 (*t*, C(7)); 71.94 (*d*, C(8)); 136.76 (*d*, C(9)); 125.25 (*d*, C(10)); 128.65 (*d*, C(11)); 130.31 (*d*, C(12) or C(15)); 26.53 (*t*, C(13)); 127.63 (*d*, C(14)); 130.96 (*d*, C(15) or C(12)); 27.57 (*t*, C(16)); 29.69 (*t*, C(17)); 31.81 (*t*, C(18)); 22.96 (*t*, C(19)); 14.31 (*q*, C(20)). MS: 330 (20, $M^+ - 18$), 193 (50), 91 (100).

15. *Transformation of (+)-***17** *into (+)-***18**. Following the procedure of *Exper. 7*, (+)-**17** was transformed in 87% yield into (+)-**18**. ^{1}H-NMR and [α]: matching those above.

16. *Silylations.* Compounds (+)-**3**, (+)-**13**, or (+)-**18** (*ca.* 1 mg) were, in turn, mixed with an excess of *sylon-BTZ (Supelco)* and a drop of pyridine in a dry atmosphere. After 5 min, MeOH was added and the mixture evaporated and analyzed by MS (*m/z* 263 (30), 263 (18), and 265 (16) for **4**, **14**, and **19**, resp.).

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