Supplemental Information

Macrocyclic oxygen transfer in conversion of fatty acid hydroperoxide to a single species of triol in physiological saline

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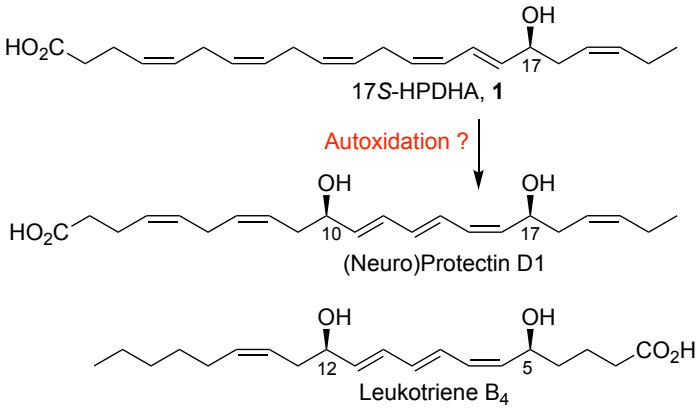
Supplemental text: α-Tocopherol-controlled autoxidation of 17*S*-HPDHA, 1

The assigned structure of (Neuro)Protectin D1 (PD1 or NPD1), (Scheme S1), has been challenged over the years based on its original characterization as a product of soybean lipoxygenase activity (which goes against precedent) and close co-chromatography of synthetic PD1 with several other dihydroxy isomers of DHA.[1-4] To examine its potential non-enzymic formation from 17*S*-HPDHA (**1**), we initiated a study of the α-tocopherol-controlled autoxidation of **1,** along the lines of other polyunsaturated fatty acid substrates,[5-7] specifically to examine the potential formation of (neuro)protectin D1, a 10*R*,17*S* diol and structural analogue of leukotriene B_4, Scheme S1,[8] 17*S*-HPDHA **1** (1 mg) was mixed with α-tocopherol (50 % by weight) and incubated overnight at room temperature as the neat oil. α-Tocopherol (vitamin E) acts as a radical trap of the initially-formed fatty acid peroxyl radicals as hydroperoxides.[5] The UV spectrum of the reaction indicated an approximately 25% decrease in the 237 nm conjugated diene chromophore of the starting material with a corresponding increase in absorbance around 270 nm. The sample was reduced with $NaBH_4$ and analyzed by RP- and NP-HPLC, separating pairs of diastereomers of 10,17-dihydroxy derivatives with all-*trans* or *trans*,*cis*,*trans* conjugated trienes as expected from vitamin E-controlled autoxidation of other polyunsaturated fatty acid hydroperoxides.[6, 7] To examine the potential formation of a 10,17-dihydroxy derivative with *trans*,*trans*,*cis* conjugated triene (i.e. matching Protectin D1[8-10]), synthetic PD1 was chromatographed with aliquots of the reduced reaction products. On RP-HPLC, PD1 co-chromatographs with other 10,17-dihydroxy isomers and is not clearly distinguished other than by advanced LC-MS methodologies (c.f.[4, 11]). However, on NP-HPLC PD1 is clearly resolved from other isomers and was found to be absent from the 17-HPDHA autoxidation (Supplement, Figure S1). Further analyses were not conducted and we switched to examining the fate of 17-HPDHA upon incubation in phosphate-buffered saline (PBS).

Supplementary Scheme S1



Supplemental Figure S1:
NP-HPLC of $NaBH_4$-reduced autoxidation products of 17*S*-HPDHA (with added PD1)

Figure S2: Proton NMR spectrum and COSY analysis of synthetic 4,5-epoxy-17-hydroxy-22:5ω3 as the methyl ester in d_6-benzene

Figure S3: Proton NMR spectrum and COSY analysis of synthetic 4,5-epoxy-17-hydroxy-22:5ω3 as the free acid in d_6-benzene

Supplemental Figure S4
RP-HPLC of γ-lactone of 4,5,17-trihydroxy-22:5ω3 control and after 5 days in PBS at 37 °C

Supplemental Figure S5:
RP-HPLC of 4,5-*cis*-epoxy-5,17-dihydroxy-22:5ω3 after incubation overnight in PBS

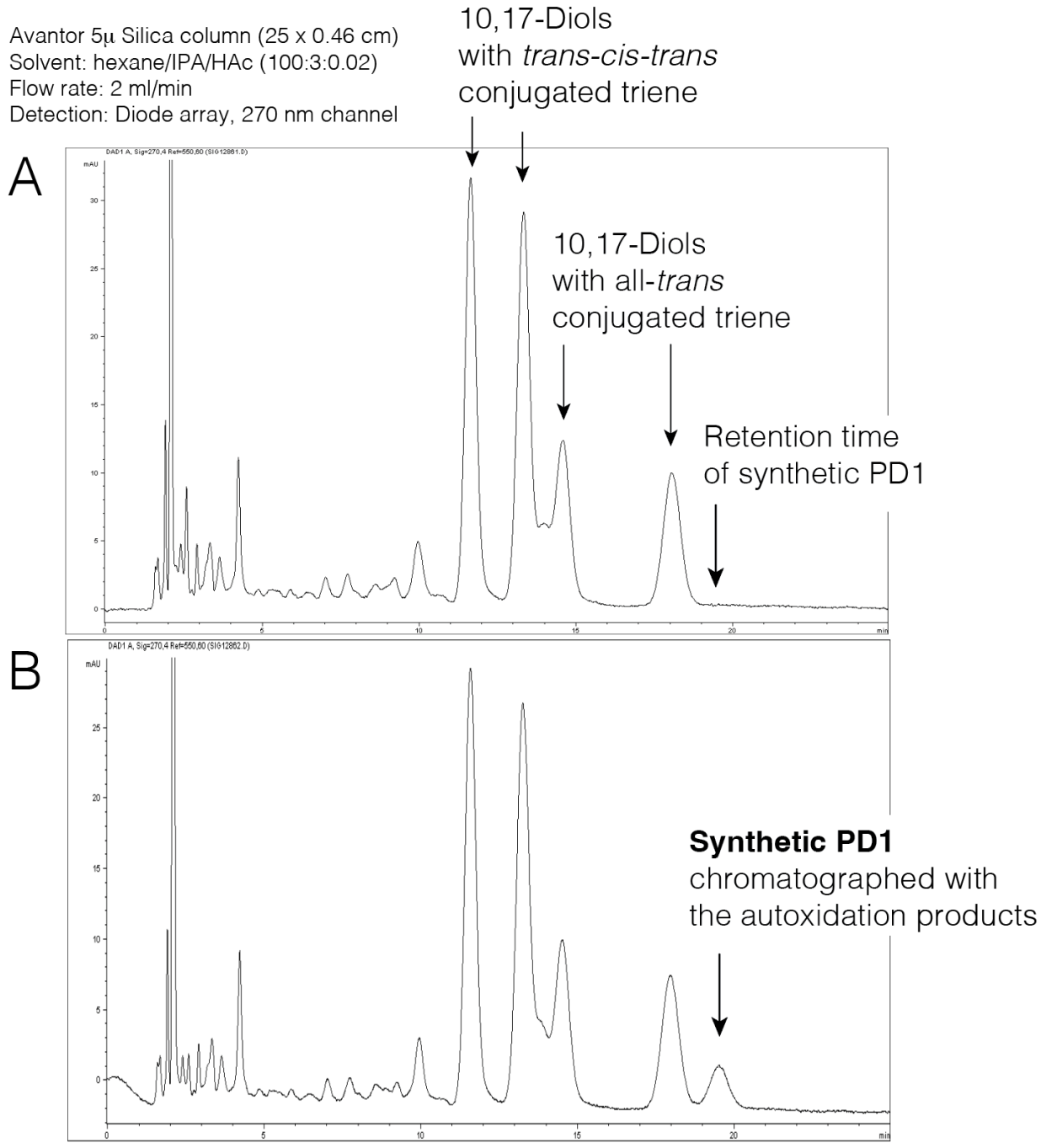
Supplemental Figure S1:

NP-HPLC of $NaBH_4$-reduced autoxidation products of 17*S*-HPDHA (with added PD1)

Aliquots of an autoxidation of 17*S*-HPDHA were reduced with $NaBH_4$ and chromatographed on an Agilent 1100 series instrument with an Avantor silica 5 μ HPLC column, a solvent system of hexane/isopropanol/glacial acetic acid (100:3:0.02 by volume), a flow rate of 2 ml/min with diode array UV detection with the 270 nm channel recording illustrated.

A: NaBH4-reduced autoxidation products

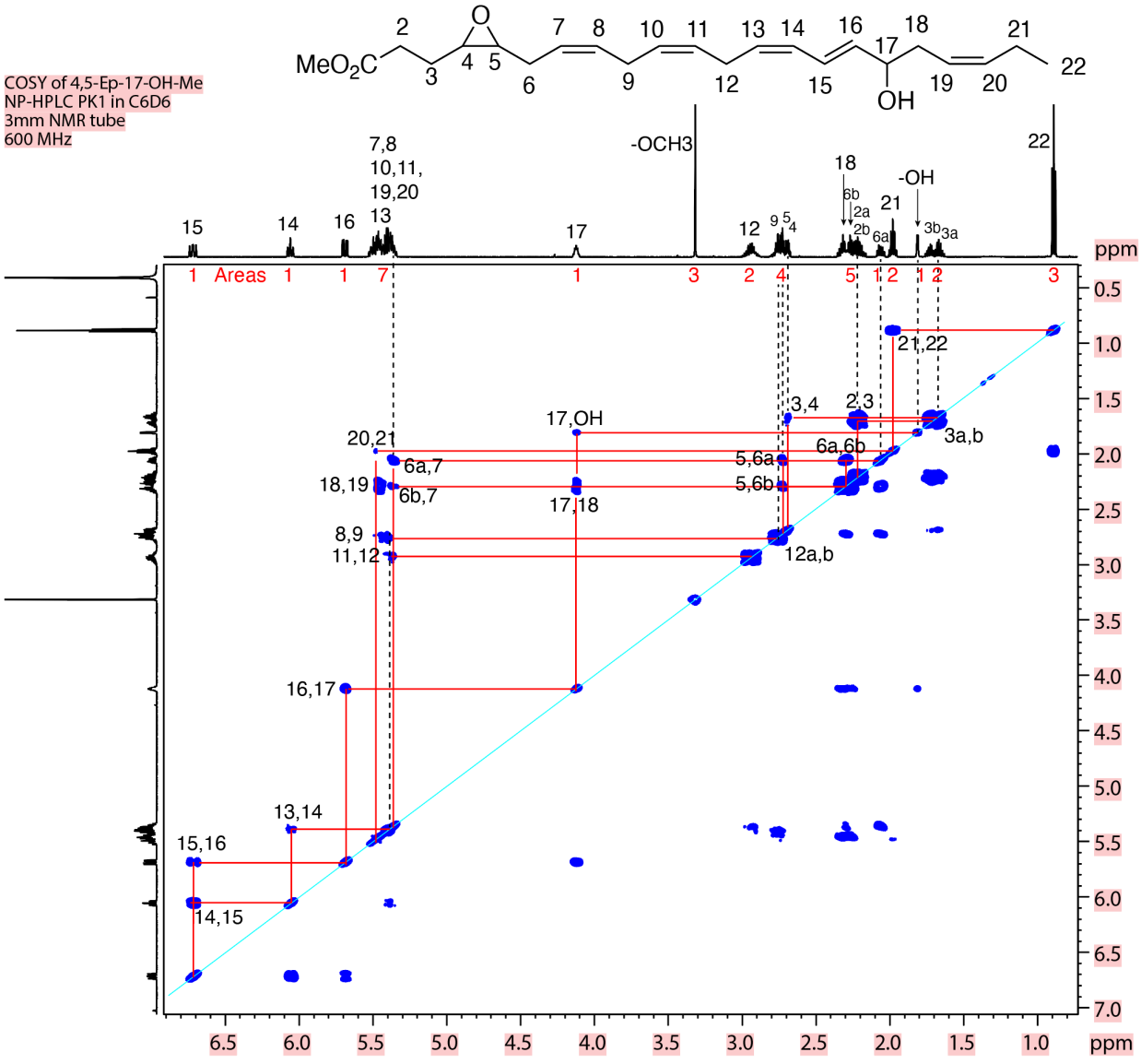
B: Another aliquot chromatographed with added synthetic PD1



Supplemental Figure S2

Proton NMR spectrum and COSY analysis of synthetic 4,5-epoxy-17-hydroxy-22:5ω3 as the methyl ester in d_6-benzene

The spectrum and COSY were recorded on the first eluting diastereomer from NP-HPLC

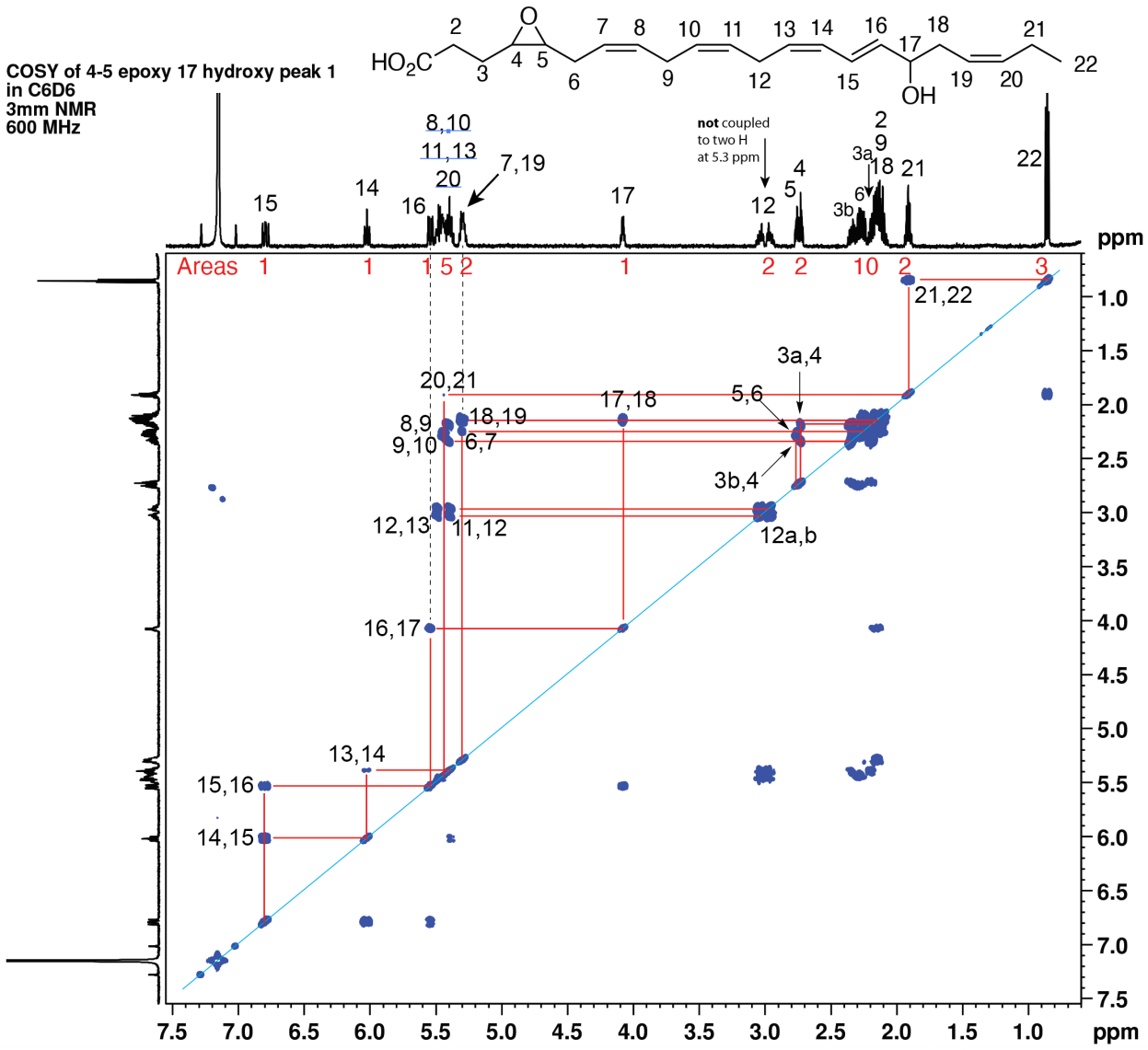


^{1}H-NMR (600 MHz, C_6D_6), Chemical shift, number of protons, multiplicity, *J*, and proton number: δ (ppm), 6.71 (1H, dd, J = 15, 11 Hz, H15); 6.05 (1H, t, J = 10.9 Hz, H14); 5.69 (1H, dd, J = 15.1, 5.8 Hz, H16); 5.34 – 5.53 (7H, m, H7, H8, H10, H11, H13, H19, H20); 4.12 (1H, m, H17); 3.31 (3H, s, OCH_3); 2.93 (2H, m, H12); 2.66 – 2.81 (4H, m, H4, H5, H9); 2.35 – 2.16 (5H, m, H2ab, H6b, H18); 2.06 (1H, m, H6a); 1.97 (2H, quintet, J = 7 Hz, H21); 1.81 (0.9H, d, -O<u>H</u> on C17); 1.76 - 1.63 (2H, m, 3a, 3b); 0.88 (3H, t, J = 7 Hz, H22).

Supplemental Figure S3

Proton NMR spectrum and COSY analysis of synthetic 4,5-epoxy-17-hydroxy-22:5ω3 as the free acid in d_6-benzene

The spectrum and COSY were recorded after KOH hydrolysis of the methyl ester derivative, extraction on an Oasis (Waters) cartridge without acidification, elution with ethyl acetate, NP-HPLC purification, and recording here of the first-eluting diastereomer.



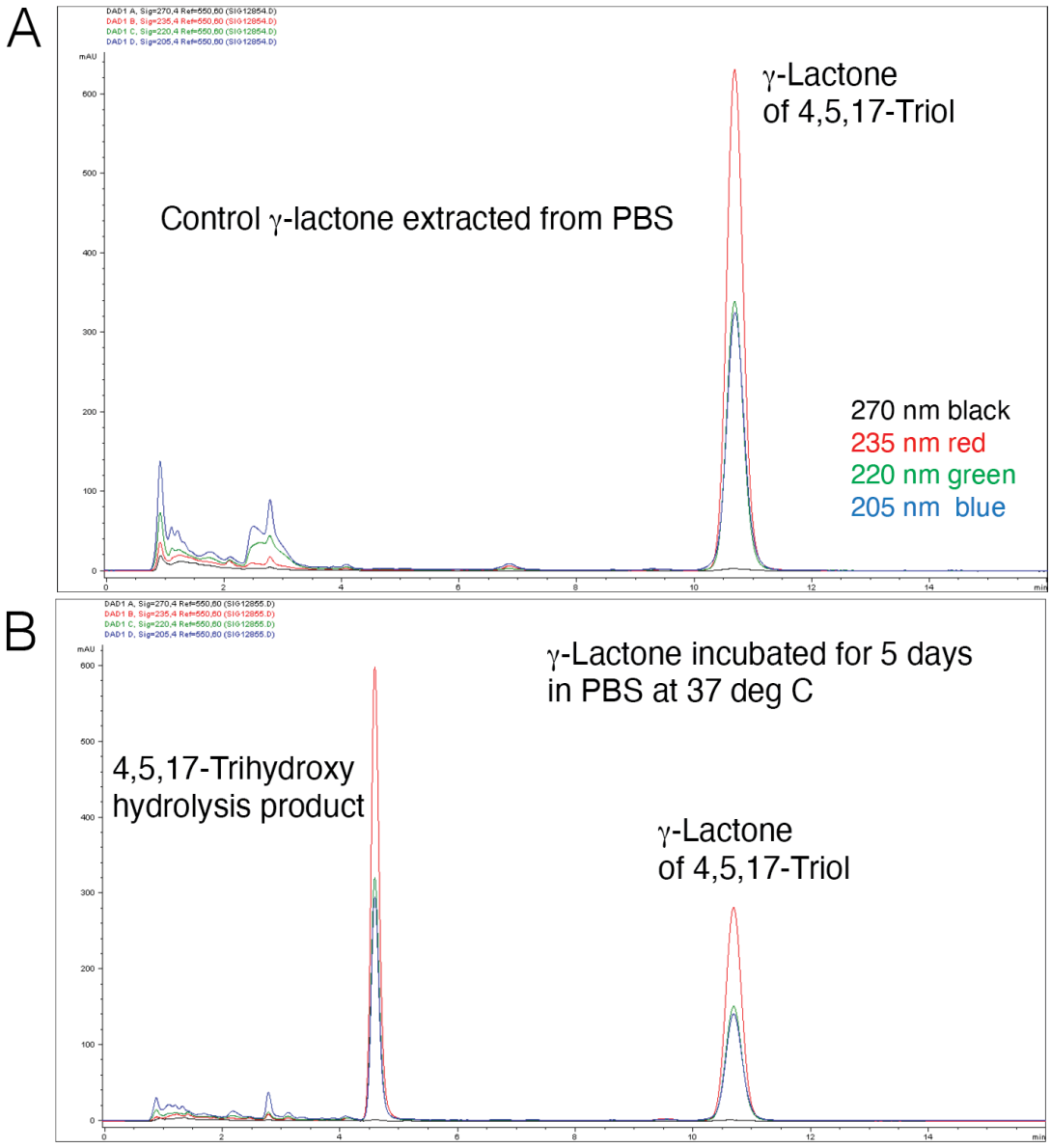
^{1}H-NMR (600 MHz, C_6D_6), Chemical shift, number of protons, multiplicity, *J*, and proton number: δ (ppm), 6.79 (1H, dd, J = 15.2, 11 Hz, H15); 6.02 (1H, t, J = 11 Hz, H14); 5.54 (1H, dd, J = 15.2, 5.2 Hz, H16); 5.36 – 5.51 (5H, m, H8, H10, H11, H13, H20); 5.30 (2H, m, H7, H19); 4.08 (1H, q, J = 5.9 Hz, H17); 3.04 (1H, m, H12a); 2.97 (1H, m, H12b); 2.07 – 2.22 (4H, m, H2a, H3a, H6); 2.22 – 2.07 (6H, m, H2b, H3b, H9, H18); 1.91 (2H, quintet, J = 7 Hz, H21 0.85 (3H, t, J = 7 Hz, H22).

Supplemental Figure S4

RP-HPLC of γ-lactone of 4,5,17-trihydroxy-22:5ω3 control and after 5 days in PBS at 37 °C

A: As a control, the γ-lactone of 4,5,17-trihydroxy-22:5ω3 (5 μg) was added to PBS at room temperature and extracted using an Oasis cartridge (without acidification), eluted with EtOAc, and an aliquot analyzed by RP-HPLC.

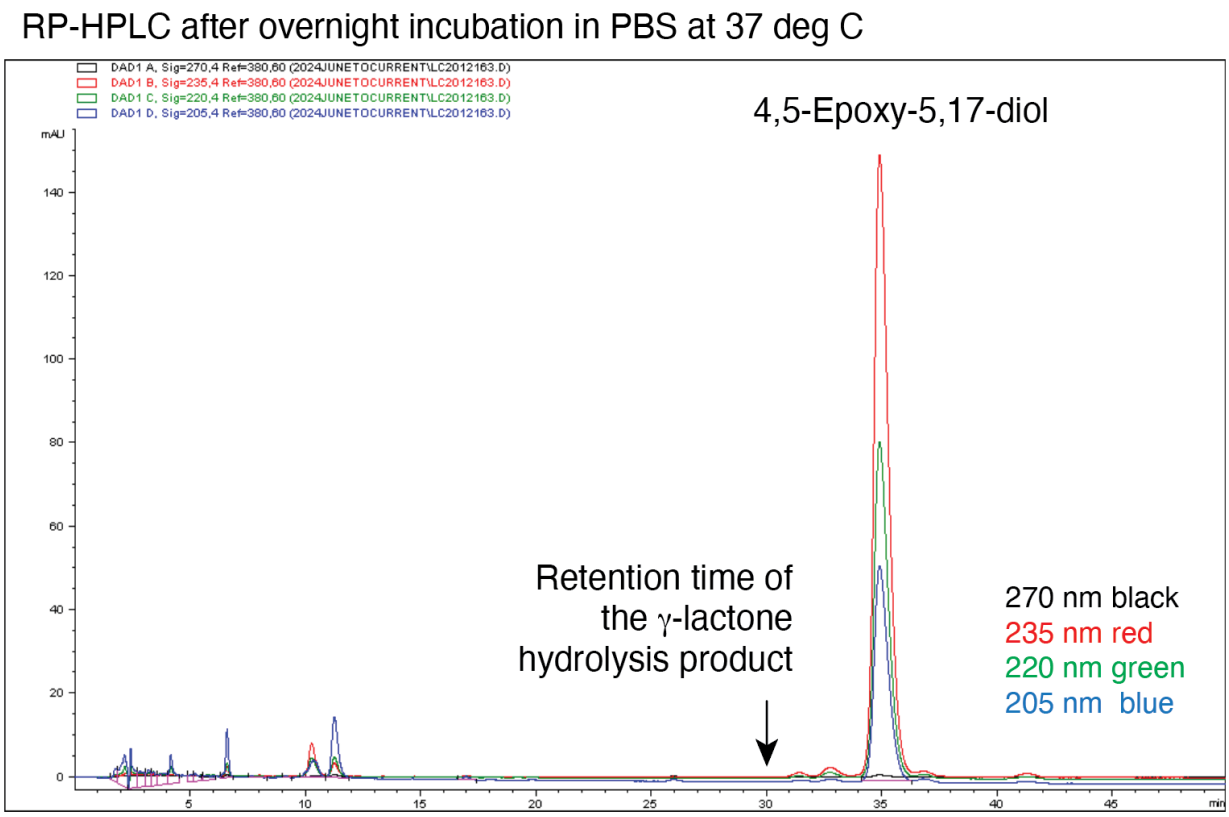
B: The γ-lactone of 4,5,17-trihydroxy-22:5ω3 (5 μg) was incubated for 5 days in PBS at 37 °C, then extracted and analyzed as above. The analyses used a Waters 5μ Symmetry column (25 x 0.46 cm) with a solvent of acetonitrile/water/glacial acetic acid (45:55:0.01 by volume) at a flow rate of 1 ml/min, with diode array detection at 205, 220, 235 and 270 nm wavelengths.



Supplemental Figure S5:

RP-HPLC of 4,5-*cis*-epoxy-5,17-dihydroxy-22:5ω3 after incubation overnight in PBS

Synthetic 4,5-epoxy-5,17-diol (10 μg) was incubated overnight in PBS at 37 °C, extracted using an Oasis cartridge (without acidification), eluted with EtOAc, and an aliquot analyzed by RP-HPLC using a Waters 5μ Symmetry column (25 x 0.46 cm) with a solvent of acetonitrile/water/glacial acetic acid (45:55:0.01 by volume) at a flow rate of 1 ml/min, with diode array detection at 205, 220, 235 and 270 nm wavelengths. There was no detectable hydrolysis to the corresponding γ-lactone derivative.



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