Supporting information for

Characterization of [8-ethyl]-chlorophyll c_3 from *Emiliania huxleyi*

Susana Álvarez,[a] Manuel Zapata,[b] José L. Garrido,*[b] and Belén Vaz,*[a]

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General methods

^{1}H-and ^{13}C-NMR spectra were measured in THF-d_8 by a Varian Inova 750 MHz spectrometer at room temperature with residual protic solvent as the internal reference. Chemical shifts (δ) are given in parts per million (ppm), and coupling constants (*J*) are given in Hertz (Hz). The proton spectra are reported as follows: δ (multiplicity, coupling constant *J*, number of protons, assignment). A set of assignments of ^{1}H signals was obtained using ^{1}H-^{1}H ROESY. All solvents were commercially available and used without further purification. Mass spectra were measured by Waters Premier liquid chromatograph coupled time-of-flight mass spectrometer (HPLC/MS-TOF) with electrospray ionization (ESI). UV spectra were measured in acetone by a Jasco V-650 Spectrophotometer.

Preparative isolation of the pigment.

Emiliania huxleyi (strain CCMP 370) was obtained from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (Bigelow Laboratory for Ocean Sciences). Mass cultures were grown in three 25 L flasks, each containing 2 L of inoculum obtained from an exponentially growing culture in the same conditions and diluted to 22 L with f/2 enriched seawater medium. Cultures were maintained at 15º C under 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of cool-white fluorescent light on a12:12 LD (light: dark) cycle, with aeration during the light period. After 12 days of growth, mass cultures were harvested by continuous centrifugation at 4ºC using a Beckman Avanti J-25 centrifuge with a JCF-Z rotor. The cell concentrate was extracted with nine times its volume of cold absolute acetone. The extract was filtered through a Whatman GF/F filter and the residue was re-extracted with cold 90% acetone until it became colorless. Pigments were quickly transferred into ethyl acetate, this solution was dried under a gentle stream of nitrogen at low temperature and the residue was resuspended in a small volume of 40% acetone and applied to the top of a glass column (40 x 2.5 cm) filled with a slurry of silanized silica gel in 40% acetone. This column was eluted with aqueous solutions containing increasing proportions of acetone. Colored bands were recovered at the column outlet and the corresponding visible spectra (350–750 nm) were measured at room temperature with a JascoV650 UV-Vis spectrophotometer and the fractions with similar spectra were combined. Two groups of combined fractions showing different chl c_3-like spectra, were subsequently purified by semipreparative HPLC using a Waters Breeze system comprised of a Waters 1525 binary pump module and double detection at 440 and 450 nm (Waters 2489 UV/Vis detector). The column was a Luna C_8 (250 x 10 mm, C_8, 5µm), and the mobile phase was an isocratic elution at 4 $mL \cdot min^{-1}$ with a mixture of eluents A:B = 40:60 (vol/vol), being eluent A methanol:acetonitrile:aqueous pyridine (0.025 M pyridine, pH 5.0 adjusted with acetic acid) 45:35:20 vol/vol/vol; and eluent B acetone. Eluted pigments were checked for homogeneity by analytical HPLC[1]. Each purified pigment was transferred into acetone by means of Waters Sep-Pak C_{18}, dried under N_2, redisolved in hexane and then purified from lipid contamination by means of semipreparative HPLC using a Sunfire prep silica column (150 x 10 mm, silica, 5µm) and a gradient from hexane to 20% 2-propanol in hexane in 20 min, at 4 $mL \cdot min^{-1}$. Lipids were monitored at 280 nm and chlorophylls at 450 nm.

[1] Zapata, M.; Rodríguez, F.; Garrido, J. L. *Mar. Ecol. Prog. Ser*. **2000**, *195*, 29-45.

Spectroscopic data

Chlorophyll-*c*$_3$

UV-Vis: λ_{max} (acetone) 625, 584, 452 nm.

^{1}H-NMR (750 MHz, THF-d_8), δ (ppm): 11.10 (s, 1H, H_5), 10.4 (s, 1H, H_{10}), 9.98 (s, 1H, H_{20}), 9.01 (d, *J*= 15.9 Hz, 1H, $H_{17'}$), 8.46 (dd, *J*= 11.9 and 18.3 Hz, 1H, $H_{8'}$), 8.29 (dd, *J*= 11.4 and 17.8 Hz, 1H, $H_{3'}$), 7.01 (s, 1H, $H_{13'}$), 6.74 (d, *J*= 15.8 Hz, 1H, $H_{17''}$), 6.51 (d, *J*= 17.9 Hz, 1H, $H_{3''}$), 6.40 (d, *J*= 18.0 Hz, 1H, $H_{8''}$), 6.30 (d, *J*= 11.6 Hz, 1H, $H_{8''}$), 6.11 (d, *J*= 11.4 Hz, $H_{3''}$), 4.31 (s, 3H, $C_{7'}$-CO_2CH_3), 3.80 (s, 3H, C_{12}-CH_3), 3.77 (s, 3H, $C_{13''}$-CO_2CH_3), 3.70 (s, 3H, C_{18}-CH_3), 3.66 (s, 3H, C_2-CH_3) ppm.

MS (ESI), *m/z*: 652 $(M-H)^-$

[8-ethyl]-Chlorophyll *c*$_3$

UV-Vis: λ_{max} (acetone) 624, 580, 446 nm

^{1}H-NMR (750 MHz, THF-d_8), δ (ppm): 11.10 (s, 1H, H_5), 10.32 (s, 1H, H_{10}), 10.01 (s, 1H, H_{20}), 9.08 (d, *J*= 15.9 Hz, 1H, $H_{17'}$), 8.29 (dd, *J*= 17.9 and 11.4 Hz, 1H, $H_{3'}$), 7.03 (s, 1H, $H_{13'}$), 6.74 (d, *J*= 15.8 Hz, 1H, $H_{17''}$), 6.51 (d, *J*= 17.8 Hz, 1H, $H_{3''}$), 6.11 (d, *J*= 11.6 Hz, 1H, $H_{3''}$), 4.46 (q, *J*= 7.8 Hz, 2H, $2H_{8'}$), 4.34 (s, 3H, $C_{7'}$-CO_2CH_3), 3.85 (s, 3H, C_{12}-CH_3), 3.78 (s, 3H, $C_{13''}$-CO_2CH_3), 3.72 (s, 3H, C_{18}-CH_3), 3.67 (s, 3H, C_2-CH_3), 1.88 (t, *J*= 7.8 Hz, 3H, $C_{8'}$-CH_3) ppm.

MS (ESI), *m/z*: 654 $(M-H)^-$

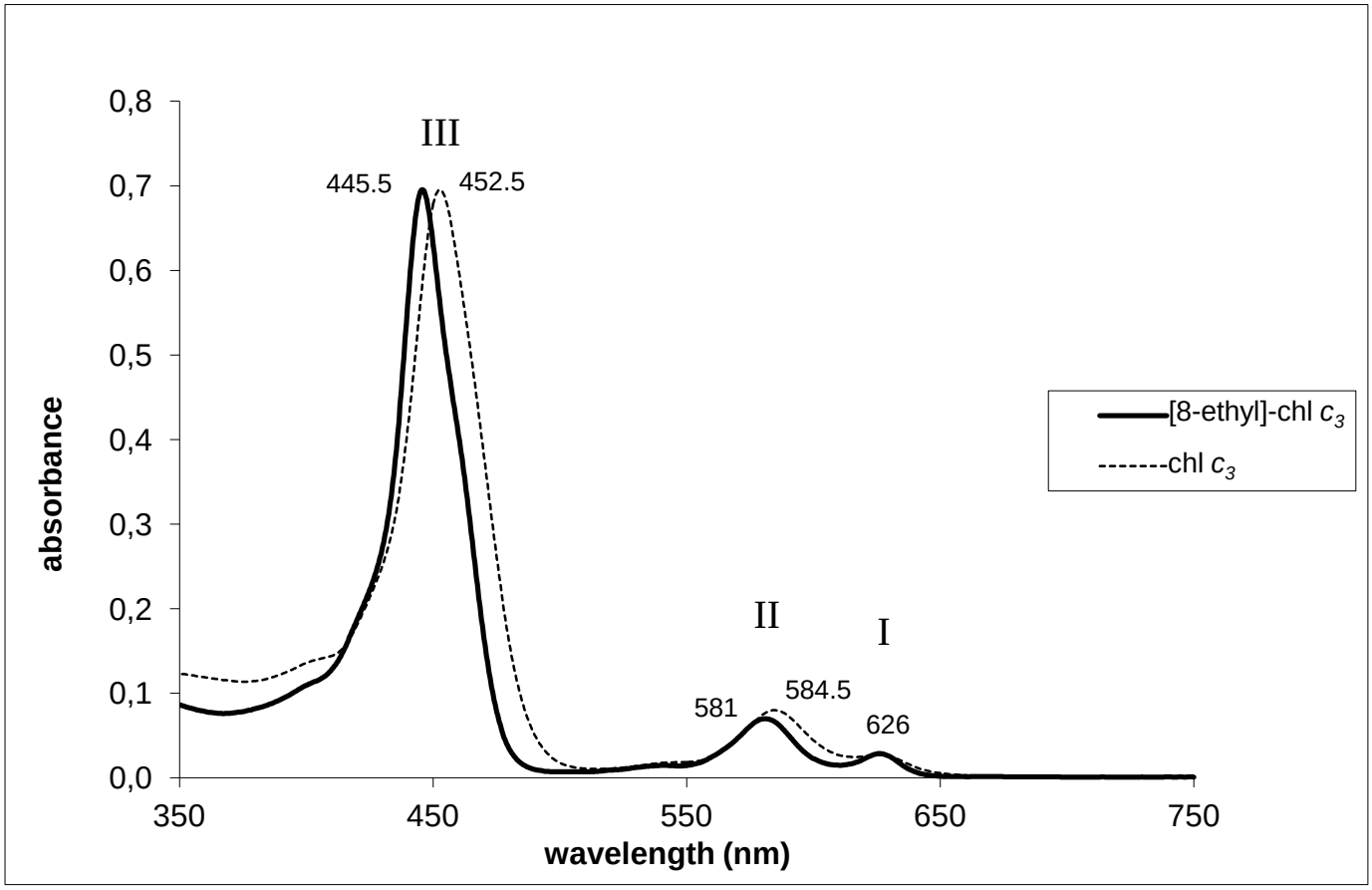

Figure S1. UV-Visible spectra of chlorophyll c_3 (dotted line) and [8-ethyl]-chlorophyll c_3 (solid line) in acetone.

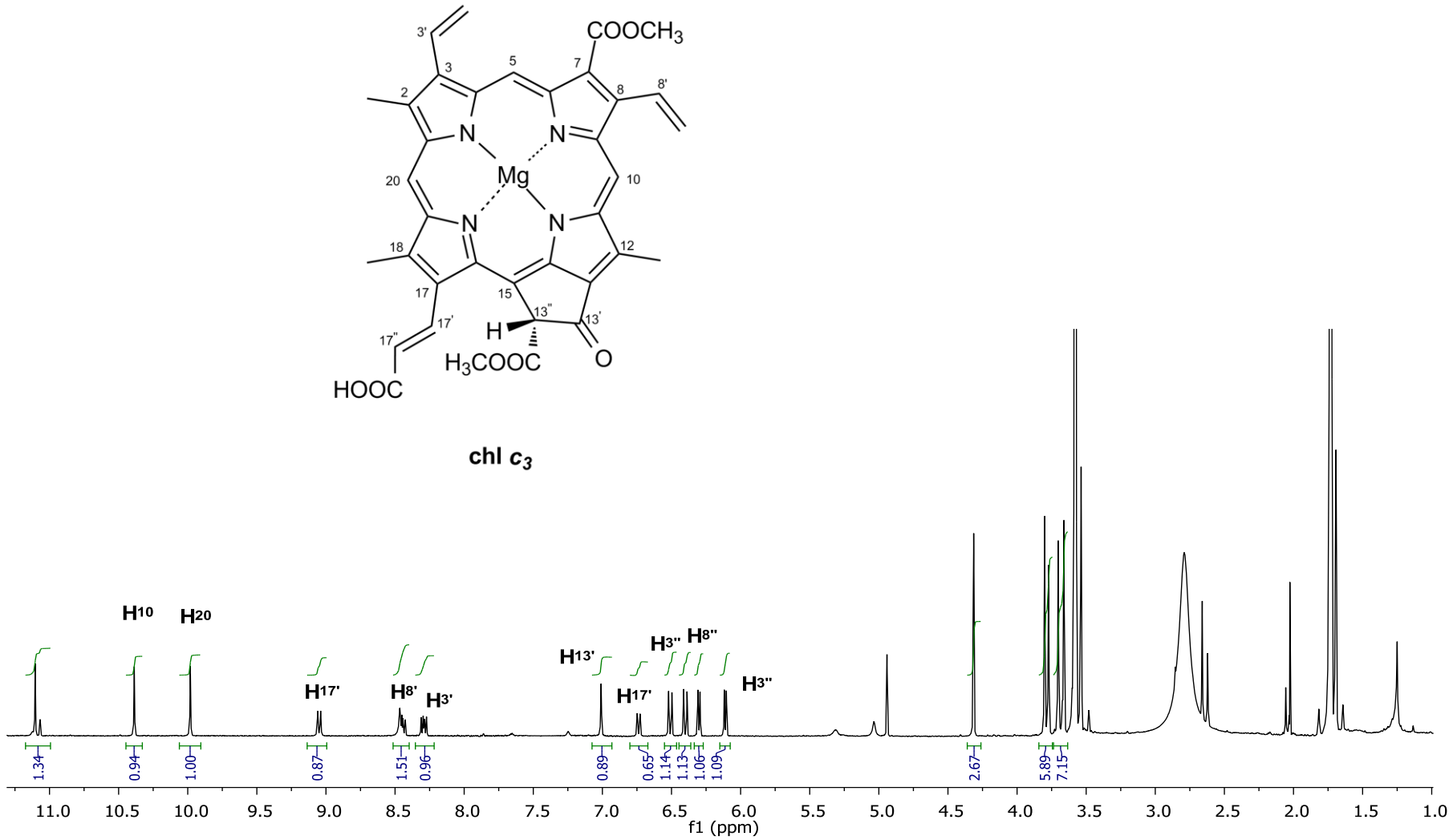

Figure S2. ^{1}H-NMR spectra of chlorophyll c_3 in THF-d_8 at room temperature.

Pyridine traces were identified coming from the eluent used in the HPLC purification method (8.54 ppm (m, 2H), 7.65 ppm (m, 1H), 7.25 ppm (m, 2H)).

It is plausible that part of thylakoid lipids have remained in the purified samples. Thus, extra peaks near 11.1 ppm maybe attributed to carboxylic functionalities present in these lipids and additional peaks other than those from the chlorophyll in the high field region of the spectrum are expected.

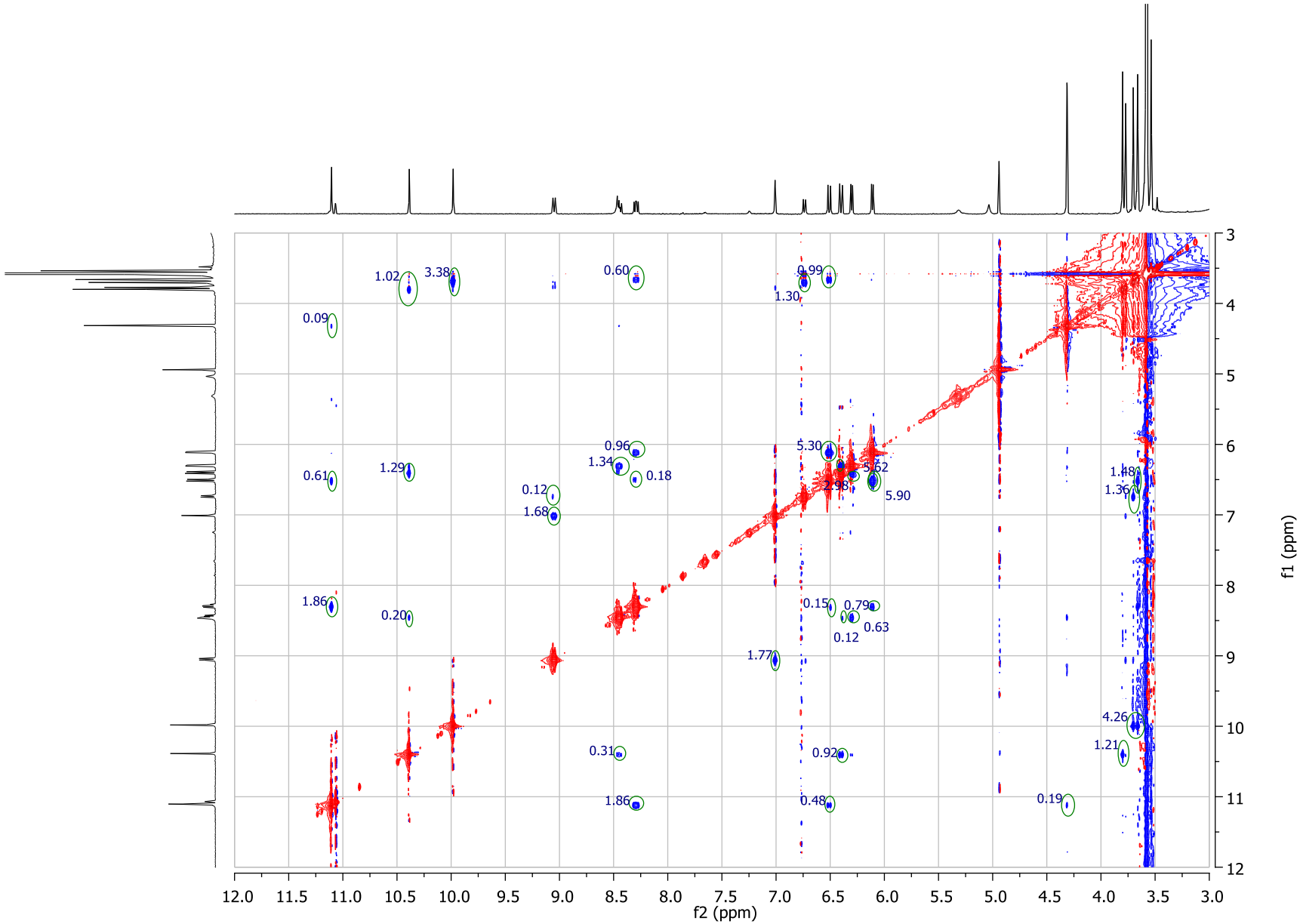

Figure S3. ROESY spectra (τ_m= 450 ms) of Chlorophyll *c*$_3$ in THF-d$_8$ at room temperature. The ROESY correlations are indicated in green circles.

Table S1: ^{1}H-NMR signal assignments and ROESY correlations for chlorophyll *c*$_3$ in THF-d$_8$ (10 mM)

	Assigment	Chemical shift δ (ppm)	Integration and Multiplicity (J_{H-H} (Hz))	Protons having ROE correlations
1	H5	11.10	1H, s	H3'', H3', OMe7'
2	H10	10.39	1H, s	H8', H8'', Me12
3	H20	9.98	1H, s	Me18, Me2
4	H17'	9.07	1H, d (15.9)	H13'
5	H8'	8.46	1H, dd (11.9 and 18.3)	OMe7', H8'', H10
6	H3'	8.29	1H, dd, (11.4 and 17.8)	H5
7	H13'	7.01	1H, s	OMe13'', H17',
8	H17''	6.74	1H, d (15.8)	Me18
9	H3''	6.51	1H, d (17.9)	Me2, H5
10	H8''	6.40	1H, d (18.0)	H8', H10
11	H8''	6.30	1H, d (11.6)	H8', H10
12	H3''	6.11	1H, d (11.4)	Me2, H5
13	OMe7'	4.31	3H, s	H5, H8'
14	Me12	3.80	3H, s	H10
15	OMe13''	3.77	3H, s	
16	Me18	3.70	3H, s	H17'', H20
17	Me2	3.66	3H, s	H20, H3''

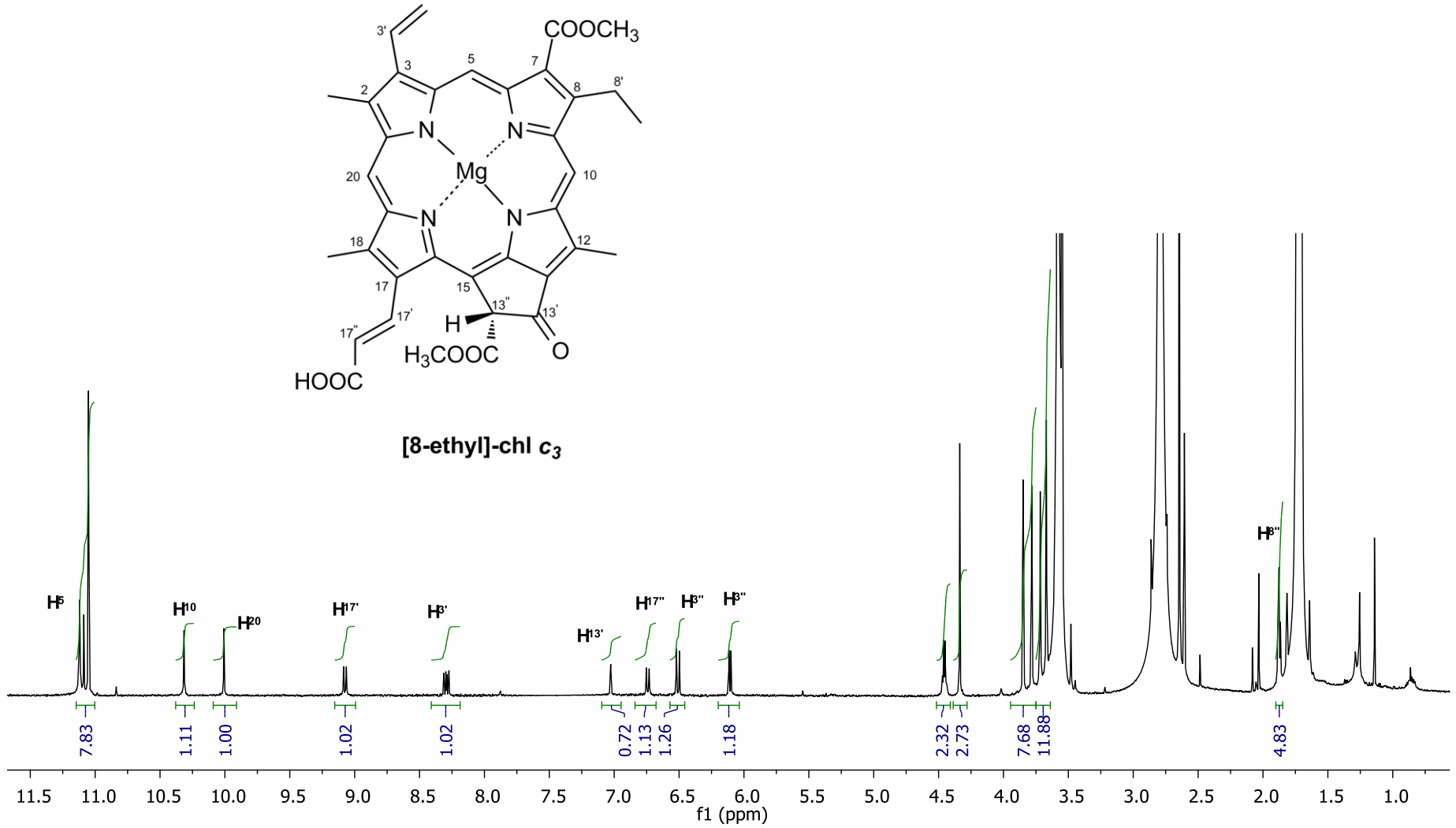

Figure S5. ^{1}H-NMR spectra of [8-ethyl]-chlorophyll c_3 in THF-d_8 at room temperature.

It is plausible that part of thylakoid lipids have remained in the purified samples. Thus, extra peaks near 11.1 ppm maybe attributed to carboxylic functionalities present in these lipids and additional peaks other than those from the chlorophyll in the high field region of the spectrum are expected.

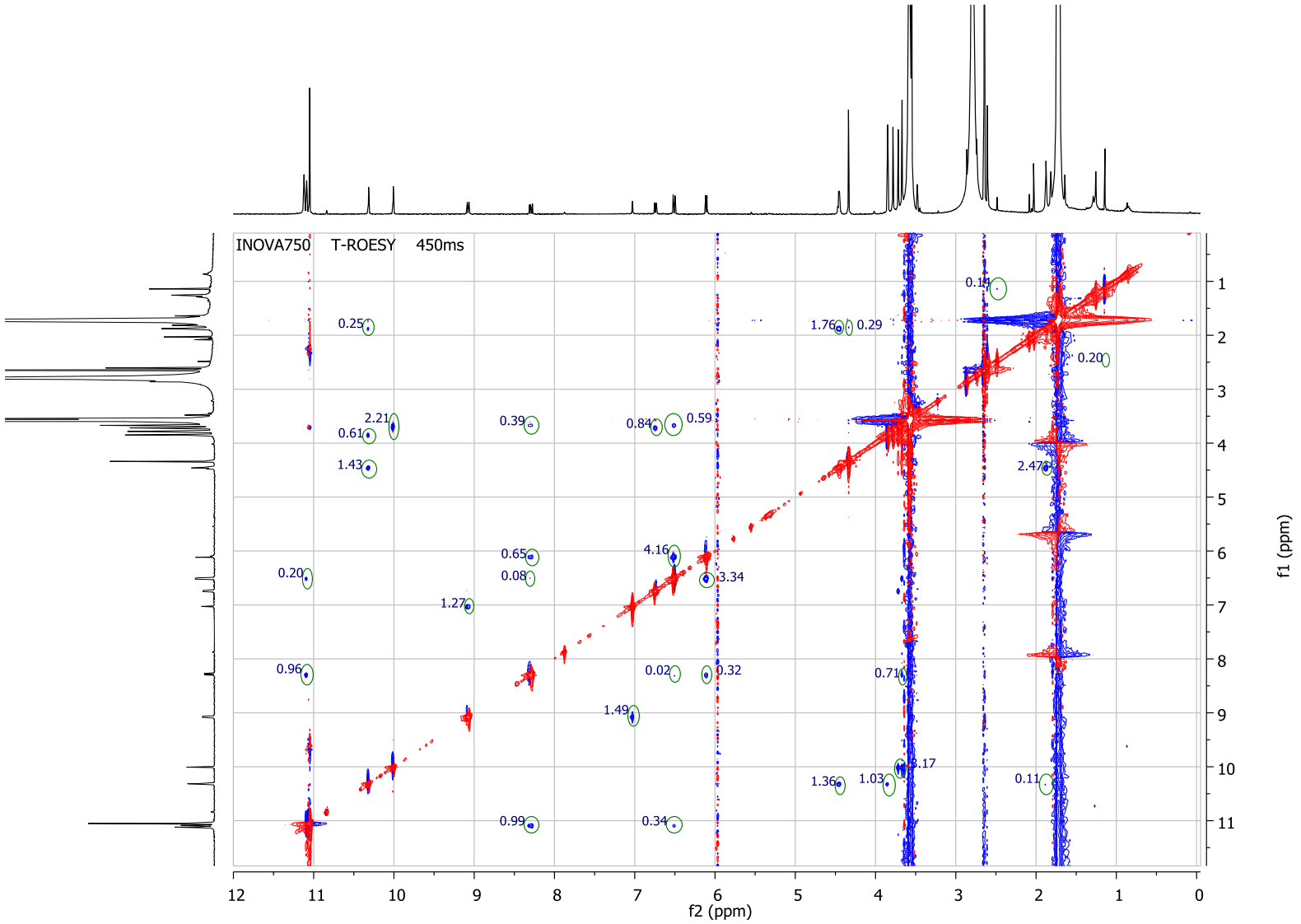

Figure S6. ROESY spectra (τ_m= 450 ms) of [8-ethyl]-chlorophyll c_3 in THF-d_8 at room temperature. The ROESY correlations are indicated in green circles.

Table S2: ^{1}H-NMR signal assignments and ROESY correlations for [8-ethyl]-chlorophyll *c*$_3$ in THF-d$_8$ (10 mM)

	Assigment	Chemical shift δ (ppm)	Integration and Multiplicity (J_{H-H} (Hz))	Protons having ROE correlations
1	H5	11.10	1H, s	H3', H3''
2	H10	10.32	1H, s	Me8'', H8', Me12
3	H20	10.01	1H, s	Me18, Me2
4	H17'	9.08	1H, d (15.9)	H13'
5	H3'	8.29	1H, dd (17.9 and 11.4)	Me2, H3'', H5
6	H13'	7.03	1H, s	OMe13'', H17'
7	H17''	6.74	1H, d (15.8)	Me18
8	H3''	6.51	1H, d (17.8)	Me2, H3', H5
9	H3''	6.11	1H, d (11.6)	Me2, H3', H5
10	2H8'	4.46	2H, q (7.8)	Me8'', H10
11	OMe7'	4.34	3H, s	Me8'', H5
12	Me12	3.85	3H, s	H10
13	OMe13''	3.78	3H, s	H13'
14	Me18	3.72	3H, s	H17'', H20
15	Me2	3.67	3H, s	H20, H3', H3''
16	Me8''	1.88	3H, t (7.8)	H10, H8', OMe7'

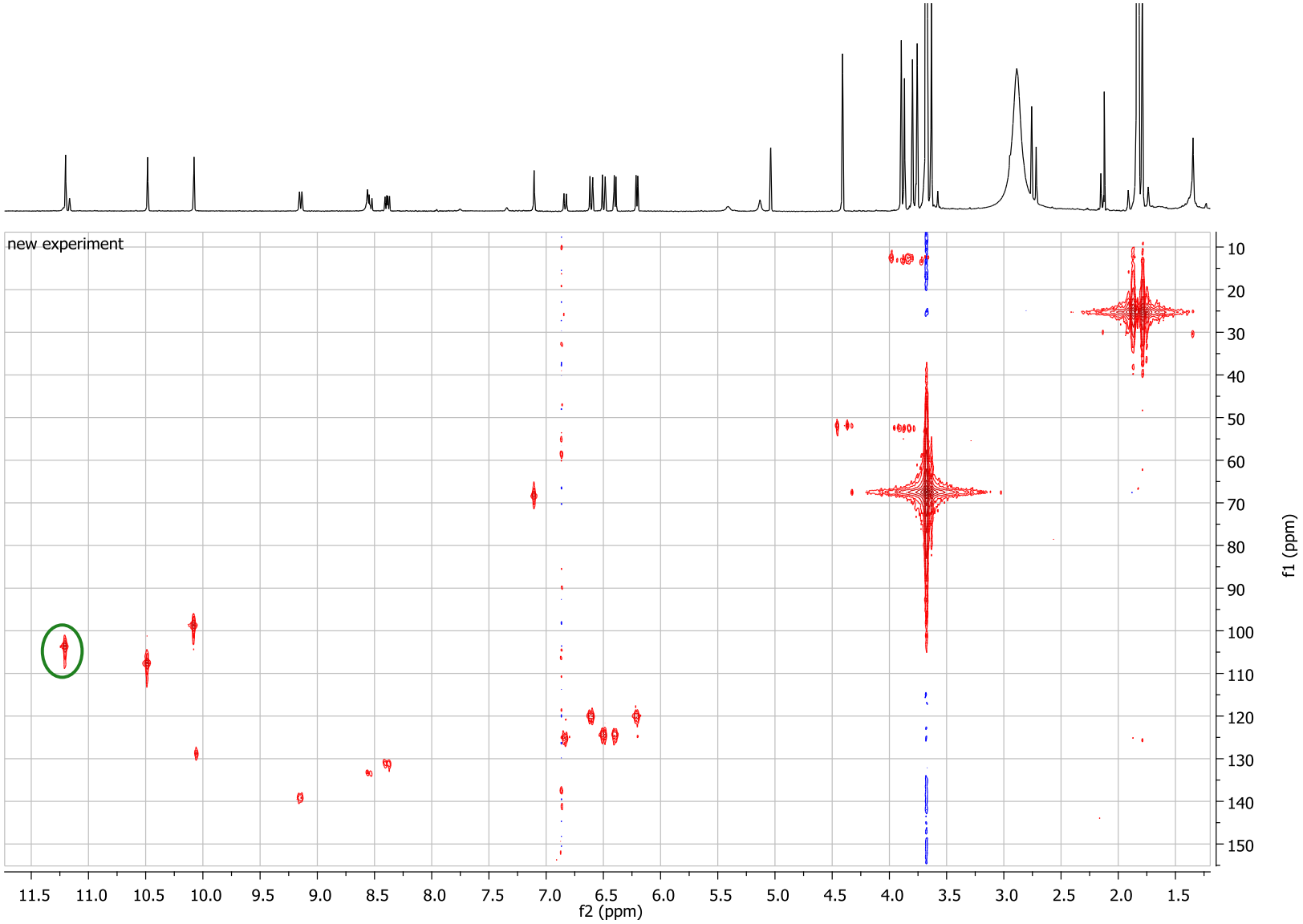

Figure S7. 2D-HSQC spectra of Chlorophyll c_3 in THF-d_8 at room temperature. The green circle shows the correlation[1] H-^{13}C for H5 giving the ^{13}C corresponding shift value of 103.6 ppm.

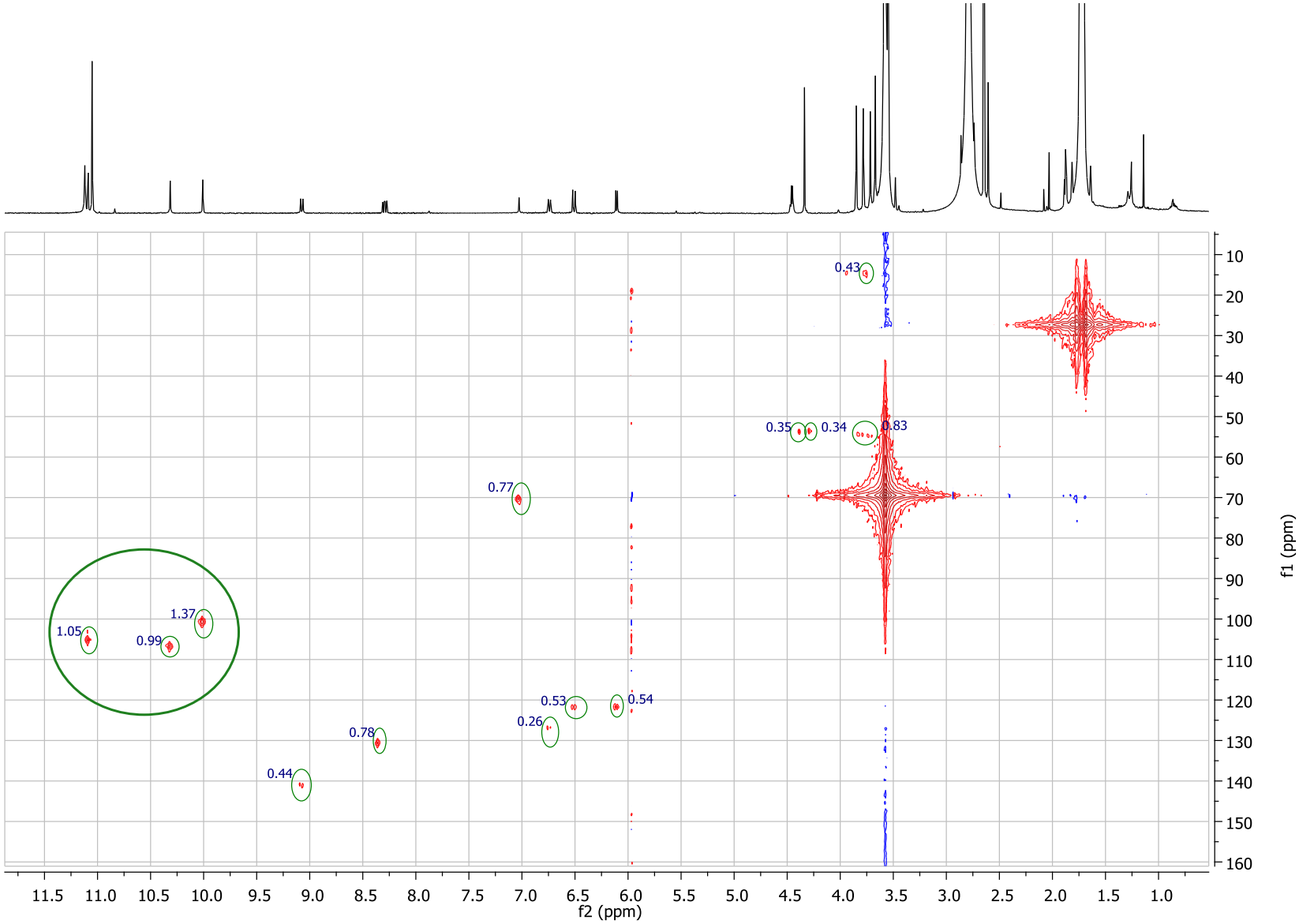

Figure S8. 2D-HSQC spectra of [8-ethyl]-chlorophyll c_3 in THF-d_8 at room temperature. The green circle shows the correlation ^{1}H-^{13}C for *meso*-CH signals.

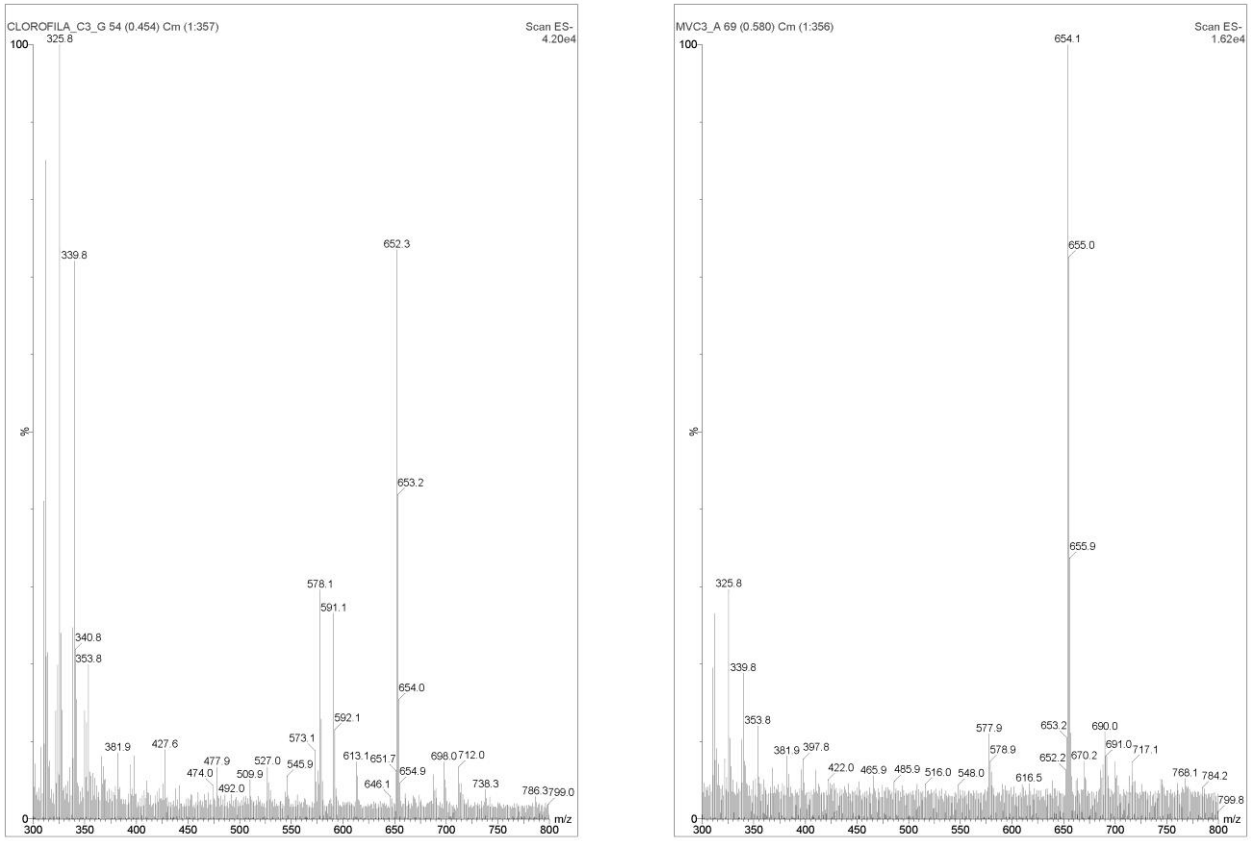

Figure S9. Mass spectra of chl c_3 (left) and [8-ethyl]-chl c_3 (right) using electrospray ionization ESI-MS in negative mode

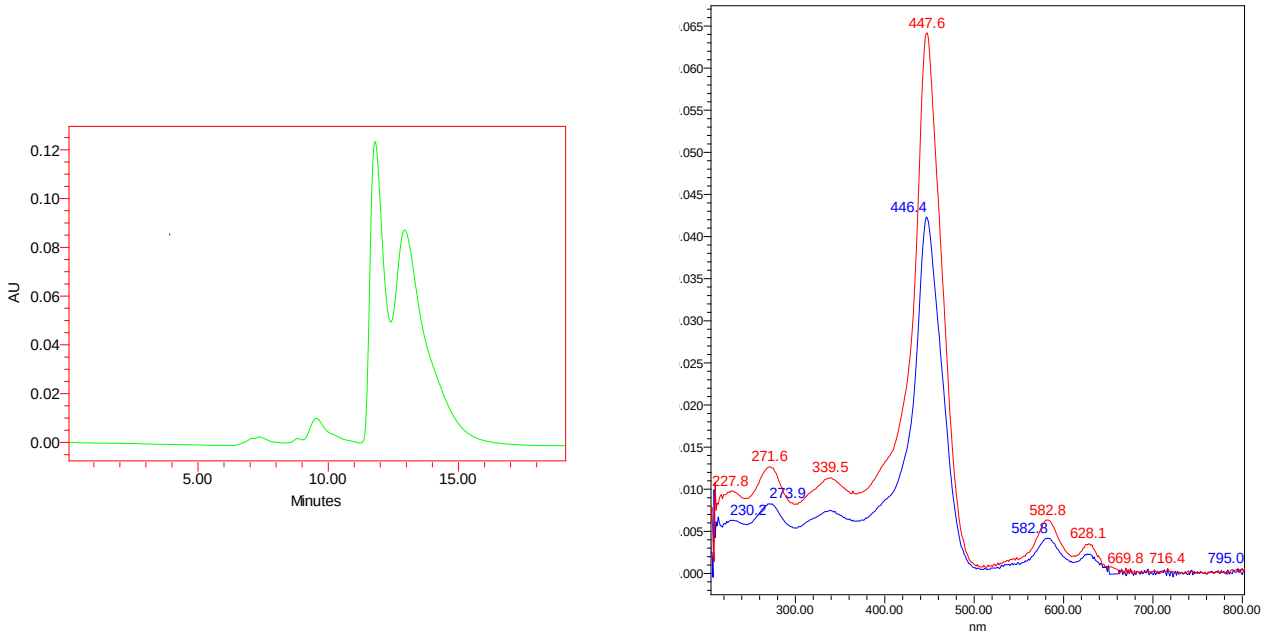

Figure S10. HPLC profile of the [8-ethyl]-chl c_3 sample extracted under basic conditions using isocratic reverse-phase chromatography in a CHIRALPAK IC column (0.5 mL/min, 1:2:2 (v/v/v) methanol : acetonitrile : aqueous 100 mM ammonium acetate solution). The two peaks were separated and they showed identical absorption.