

## Supporting Information

### Synechoxanthin: an Aromatic C<sub>40</sub> Xanthophyll is a Major Carotenoid in the Cyanobacterium *Synechococcus* sp. PCC 7002

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- Figure S1: <sup>1</sup>H NMR spectrum of synechoxanthin in DMSO-*d*<sub>6</sub>, with and without water saturation.
- Figure S2: <sup>1</sup>H NMR spectrum of synechoxanthin dimethyl ester in CD<sub>2</sub>Cl<sub>2</sub>.
- Figures S3–S5: <sup>1</sup>H-<sup>13</sup>C HMQC spectrum of synechoxanthin dimethyl ester in CD<sub>2</sub>Cl<sub>2</sub>, optimized for one-bond correlations.
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- Scheme S2: H2BC correlations.
- Figure S7–S10: Gradient selected magnitude <sup>1</sup>H-<sup>13</sup>C HMBC spectrum of synechoxanthin dimethyl ester in CD<sub>2</sub>Cl<sub>2</sub>.
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- Scheme S4: 1D-ROE connectivities.
- Figure S19: Simulation of the AA'BB' spin system (H-14,14' and H-15,15').
- Figure S20: Correlation between observed and calculated <sup>13</sup>C chemical shifts for the benzene ring.
- Table S1: Calculated <sup>13</sup>C chemical shift for the benzene ring.

Figure S1:  $^1\text{H}$  NMR spectrum of synechoxanthin in  $\text{DMSO-}d_6$ , downfield region, without (A) and with (B) saturation of the water line at 3.55 ppm. Integration of the peak at 12.75 ppm in (A) returns an intensity comparable to one of the vinylic signals. Saturation of the water line results in the saturation of the peak (B). The broad peak at 11.9 ppm arises from an impurity. All NMR data at a  $^1\text{H}$  frequency of 600.13 MHz

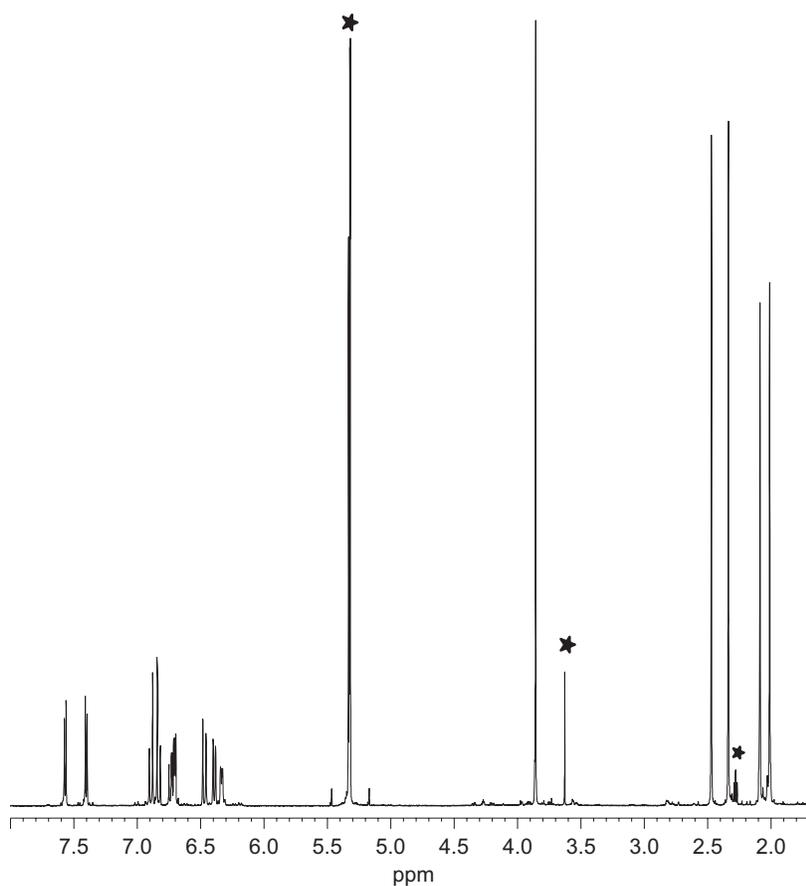
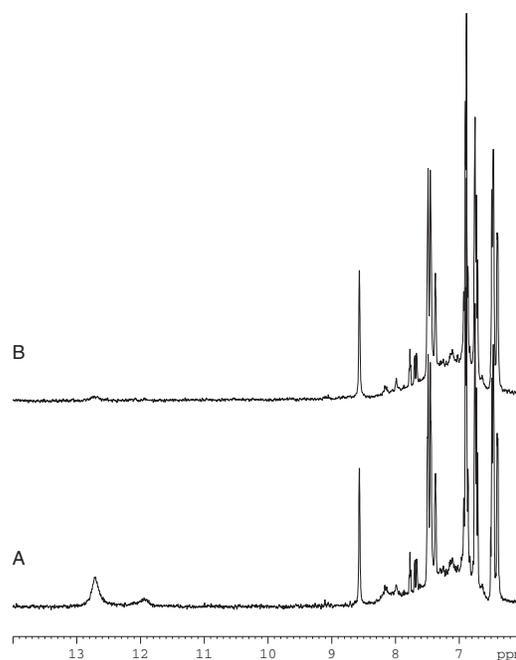


Figure S2:  $^1\text{H}$  NMR spectrum of synechoxanthin dimethyl ester in  $\text{CD}_2\text{Cl}_2$ . Stars indicate solvent impurities.  $\text{CHDCl}_2$  is at 5.32 ppm. Figure 4 in the main paper is an expansion of the aromatic/vinylic region.

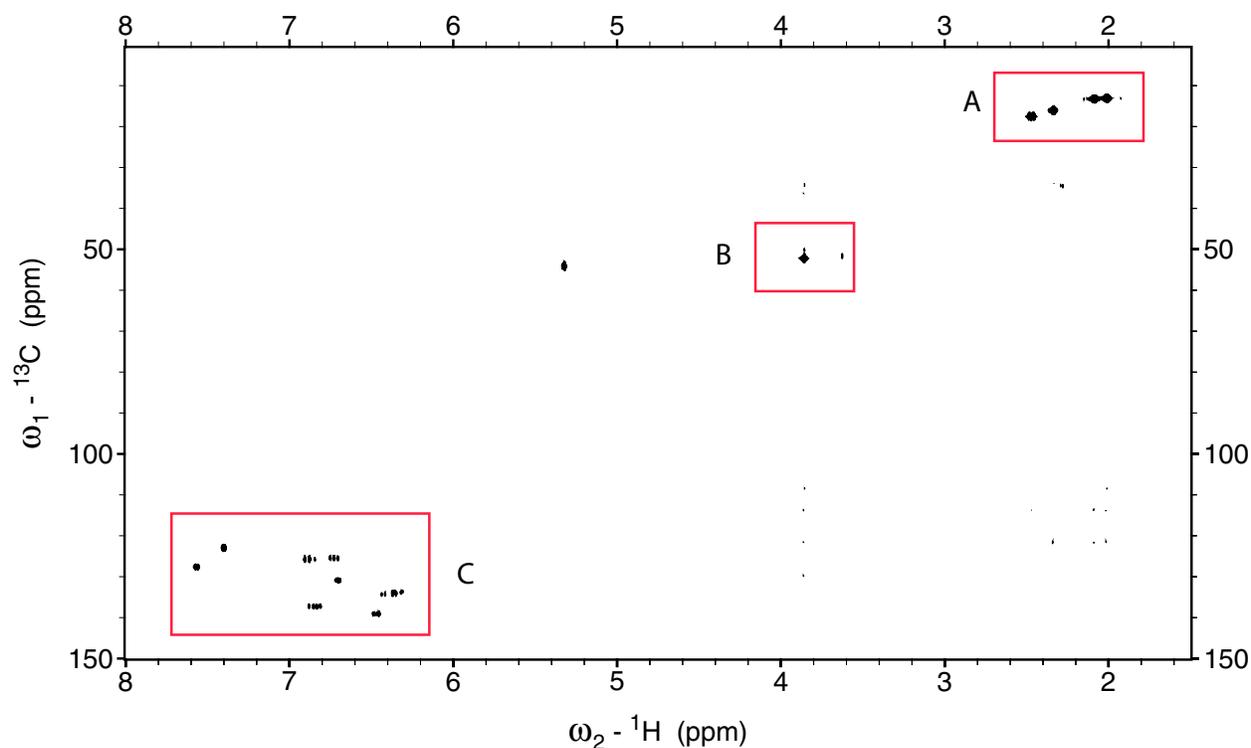


Figure S3:  $^1\text{H}$ - $^{13}\text{C}$  HMQC spectrum of synechoxanthin dimethyl ester in  $\text{CD}_2\text{Cl}_2$ , optimized for one-bond correlations. Region A contains the cross peaks from C16H<sub>3</sub>, C17H<sub>3</sub>, C19H<sub>3</sub>, and C20H<sub>3</sub>; region B contains the cross peak for the ester methyl group, C18eH<sub>3</sub>; and region C contains the cross peaks from the vinyl CH's. Peaks outside the boxes are from solvent and impurities.  $\text{CH}_2\text{Cl}_2$  is at 5.32 ppm ( $^1\text{H}$ ) and 54 ppm ( $^{13}\text{C}$ ).

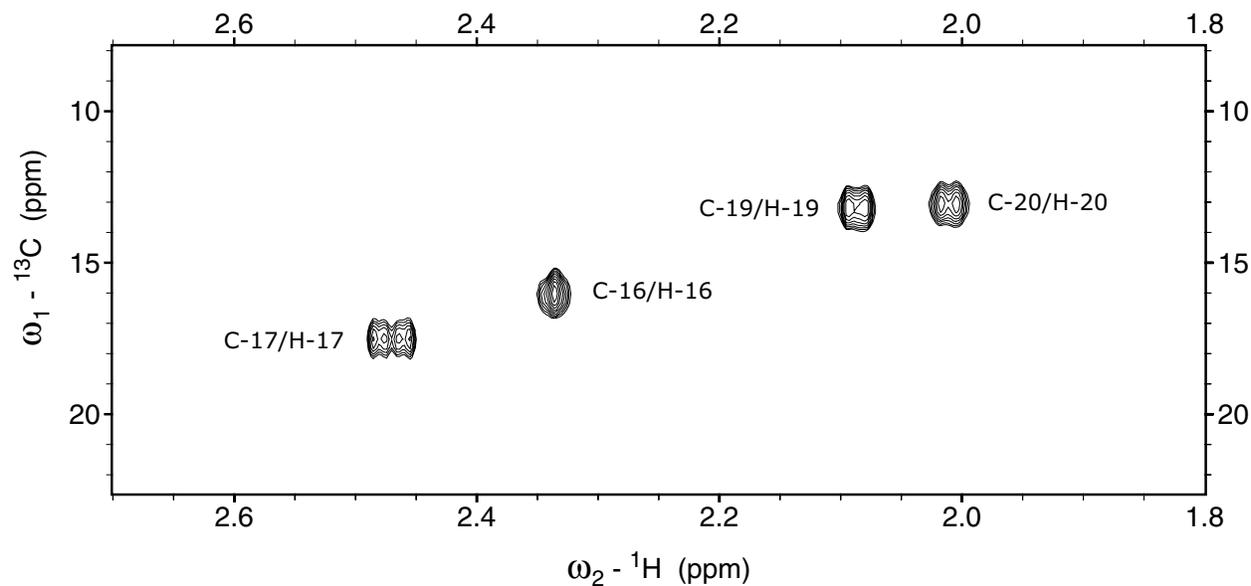


Figure S4:  $^1\text{H}$ - $^{13}\text{C}$  HMQC spectrum. Expansion of Figure S3; region A.

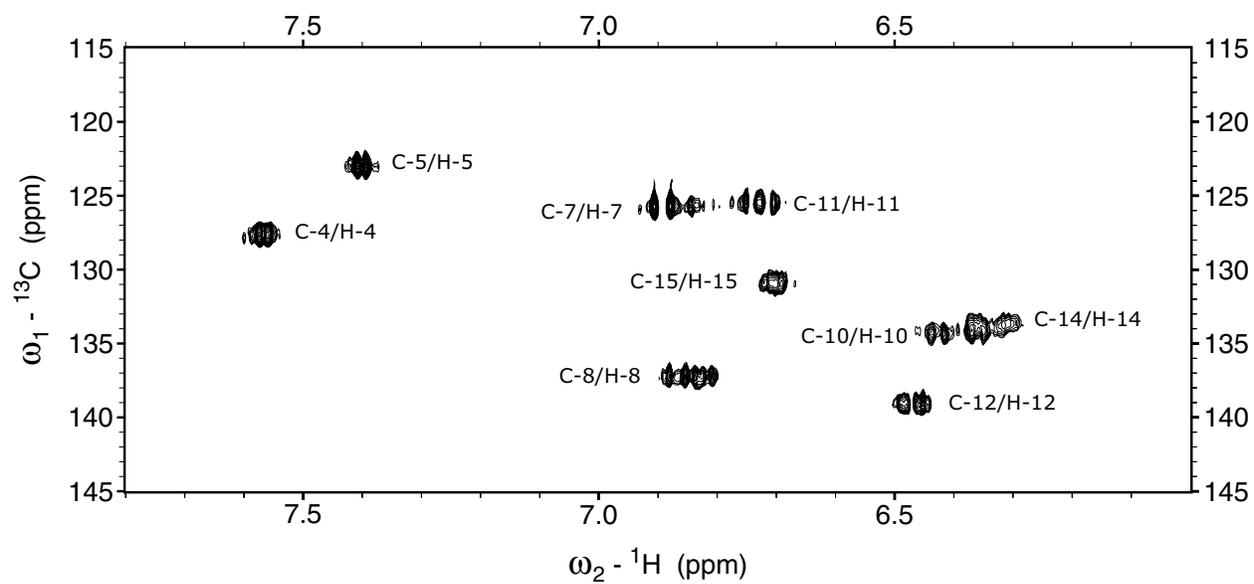
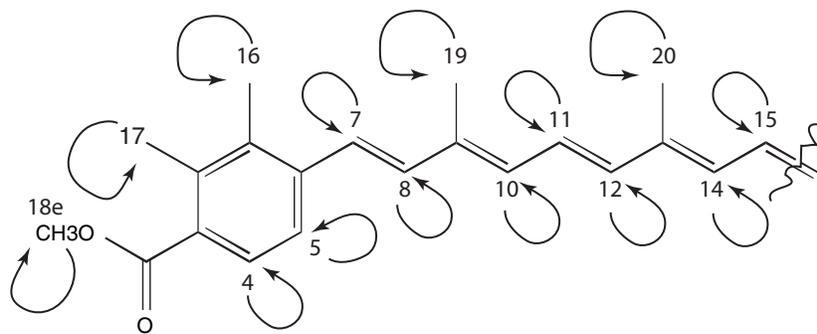


Figure S5:  $^1\text{H}$ - $^{13}\text{C}$  HMQC spectrum. Expansion of Figure S3; region C.



Scheme S1: One-bond correlations observed in the  $^1\text{H}$ - $^{13}\text{C}$  HMQC spectrum ( $^1\text{H} \rightarrow$  coupled  $^{13}\text{C}$ ).

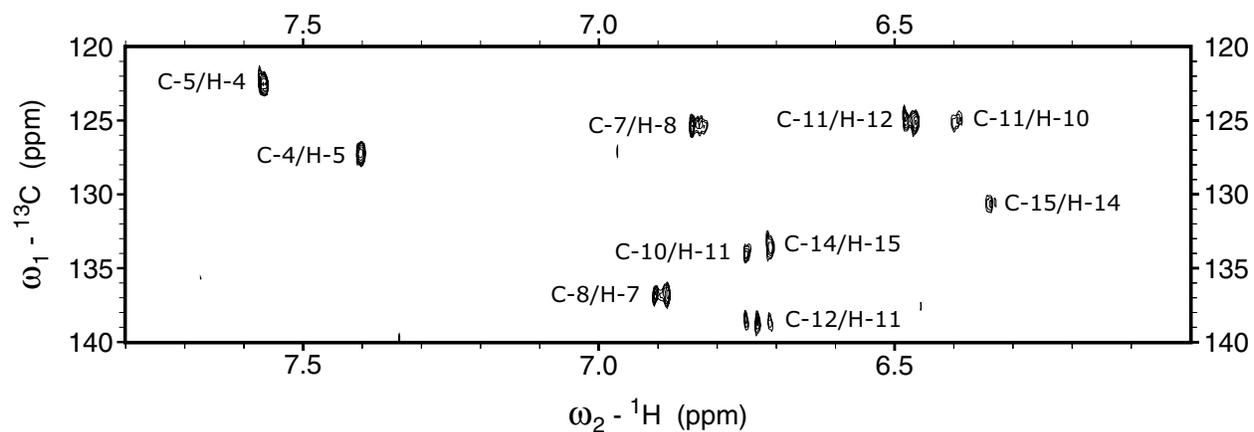
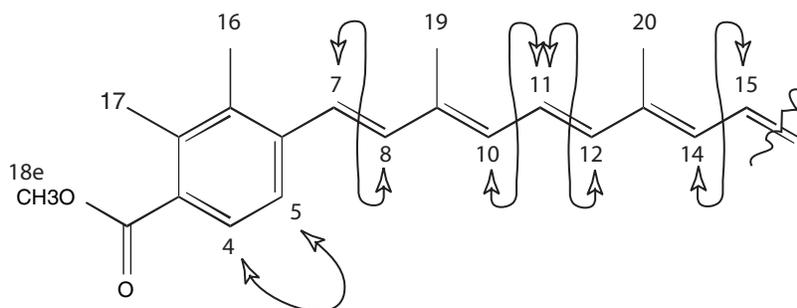


Figure S6:  $^1\text{H}$ - $^{13}\text{C}$  H2BC spectrum of synechoxanthin dimethyl ester in  $\text{CD}_2\text{Cl}_2$ .



Scheme S2: Two-bond correlations observed in the  $^1\text{H}$ - $^{13}\text{C}$  H2BC spectrum. This experiment does not provide correlations to quaternary  $^{13}\text{C}$ .

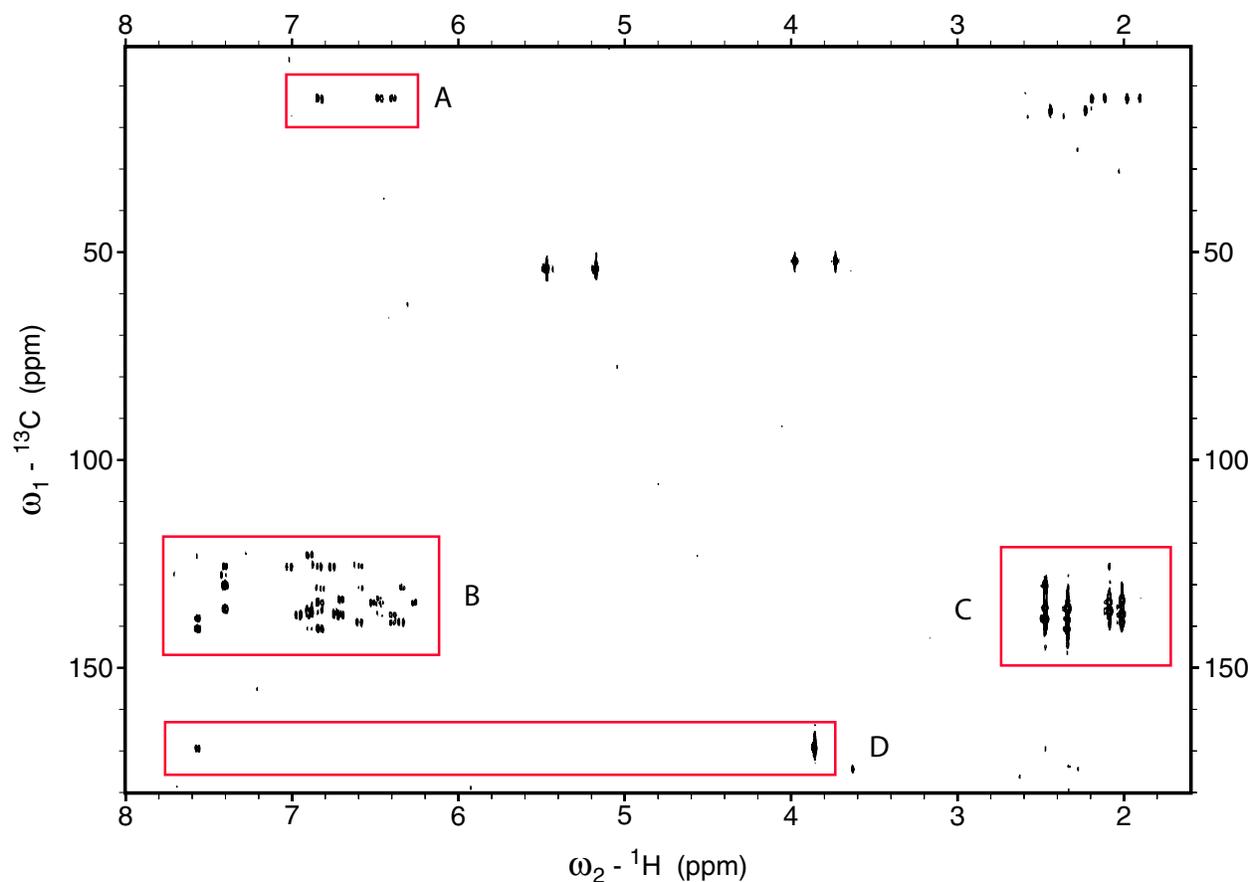


Figure S7: Gradient selected magnitude  $^1\text{H}$ - $^{13}\text{C}$  HMBC spectrum of synechoxanthin dimethyl ester in  $\text{CD}_2\text{Cl}_2$ , optimized for long-range couplings (8-10 Hz). Region A contains long-range CH to  $\text{CH}_3$  cross-peaks. Region D contains the correlations between the carbonyl CO (C-18) and  $\text{C18eH}_3$  and between C-18 and H-4, which establishes the position of the ester in the ring.

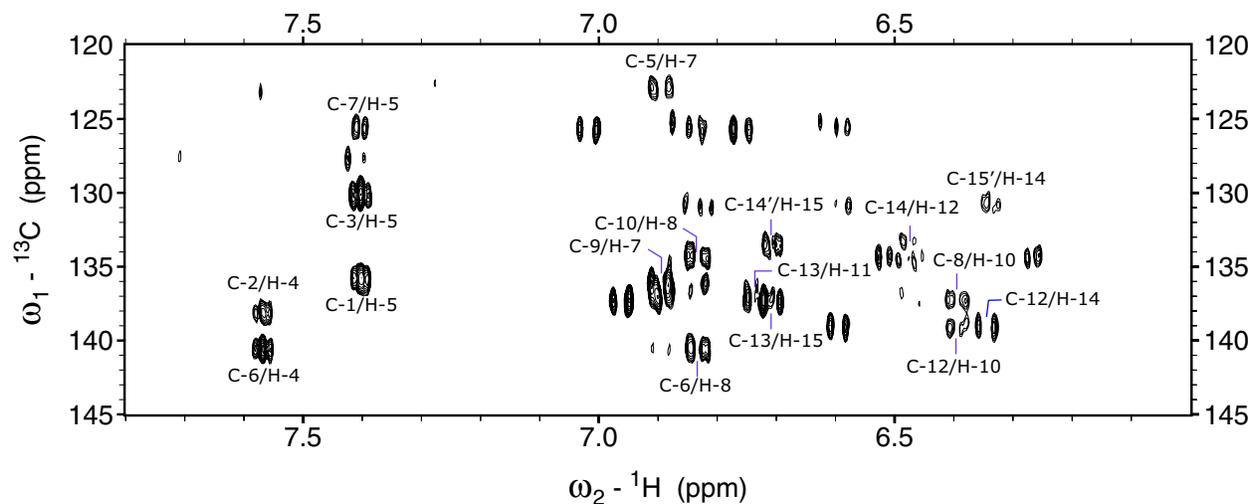


Figure S8:  $^1\text{H}$ - $^{13}\text{C}$  HMBC spectrum. Expansion of Figure S7, region B. Several cross peaks are due to low level impurities seen in the 1D spectrum shown in Figure 4.

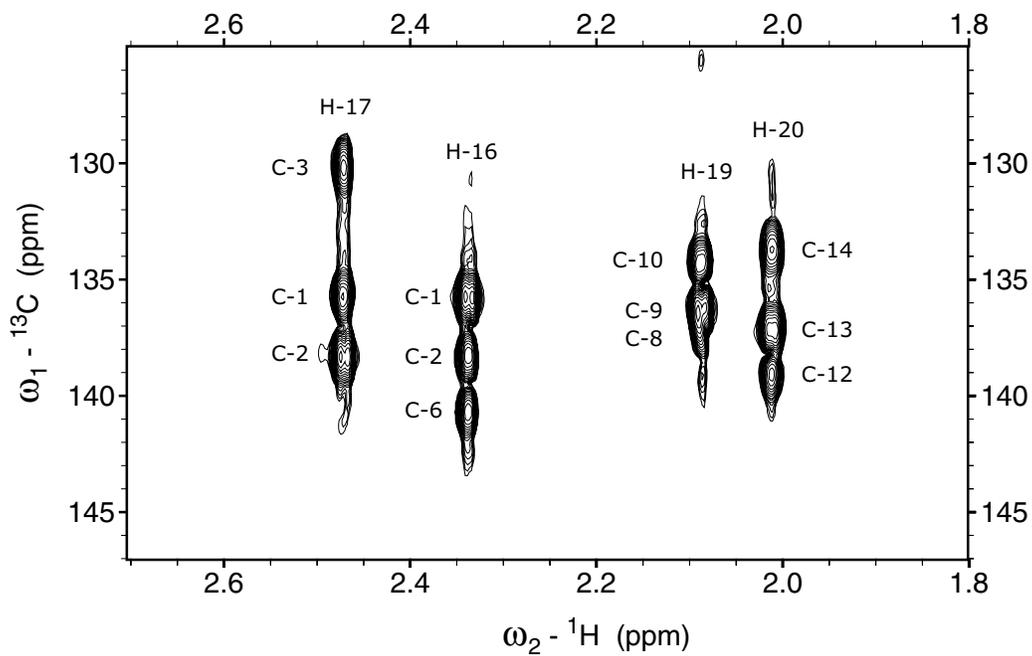


Figure S9:  $^1\text{H}$ - $^{13}\text{C}$  HMBC spectrum. Expansion of Figure S7, region C.

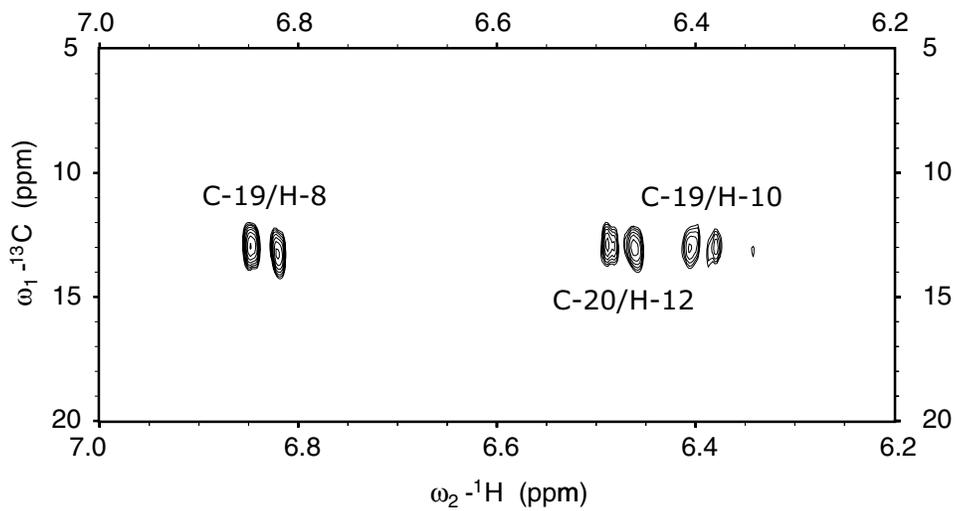
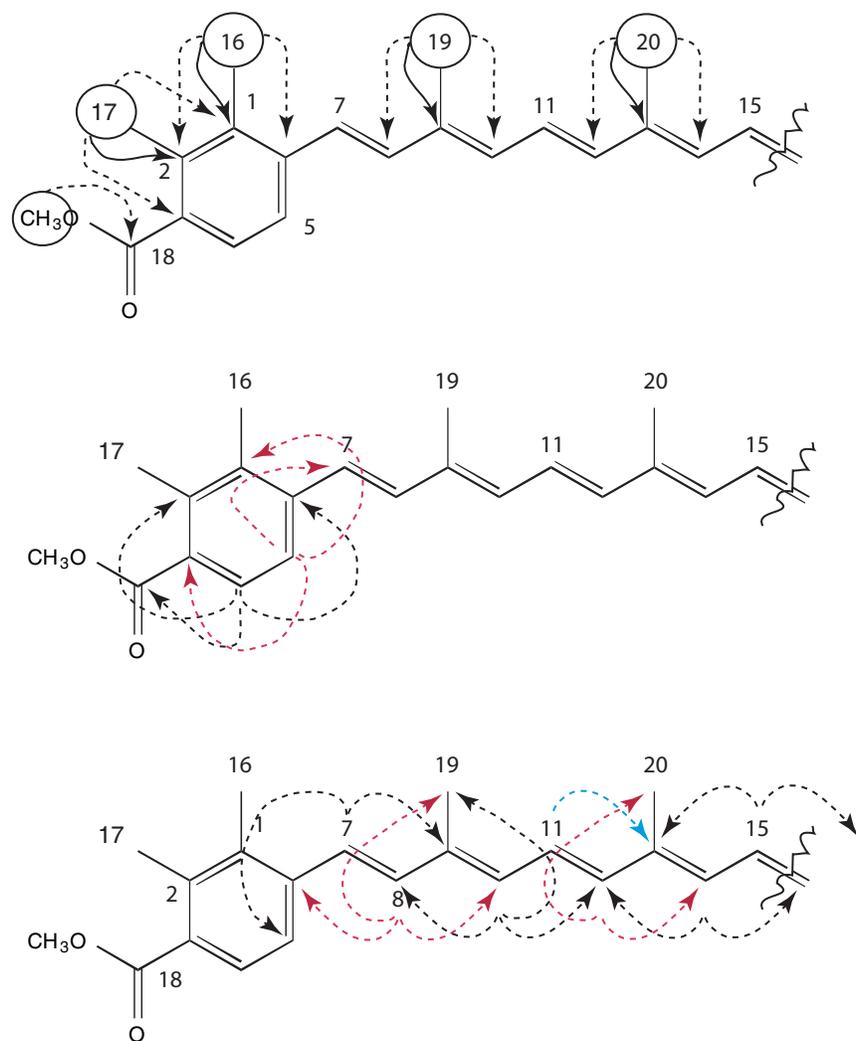


Figure S10:  $^1\text{H}$ - $^{13}\text{C}$  HMBC spectrum. Expansion of Figure S7, region A.



Scheme S3: Two- and three-bond correlations observed in the  $^1\text{H}$ - $^{13}\text{C}$  HMBC spectrum of synechoxanthin dimethyl ester in  $\text{CD}_2\text{Cl}_2$  ( $^1\text{H} \rightarrow$  coupled  $^{13}\text{C}$ ).  $^2J$  correlations are shown with solid lines,  $^3J$  correlations with dashed lines. Correlations involving methyl groups, aromatic nuclei and vinylic nuclei are shown separately. Colors are used only to clarify the scheme.

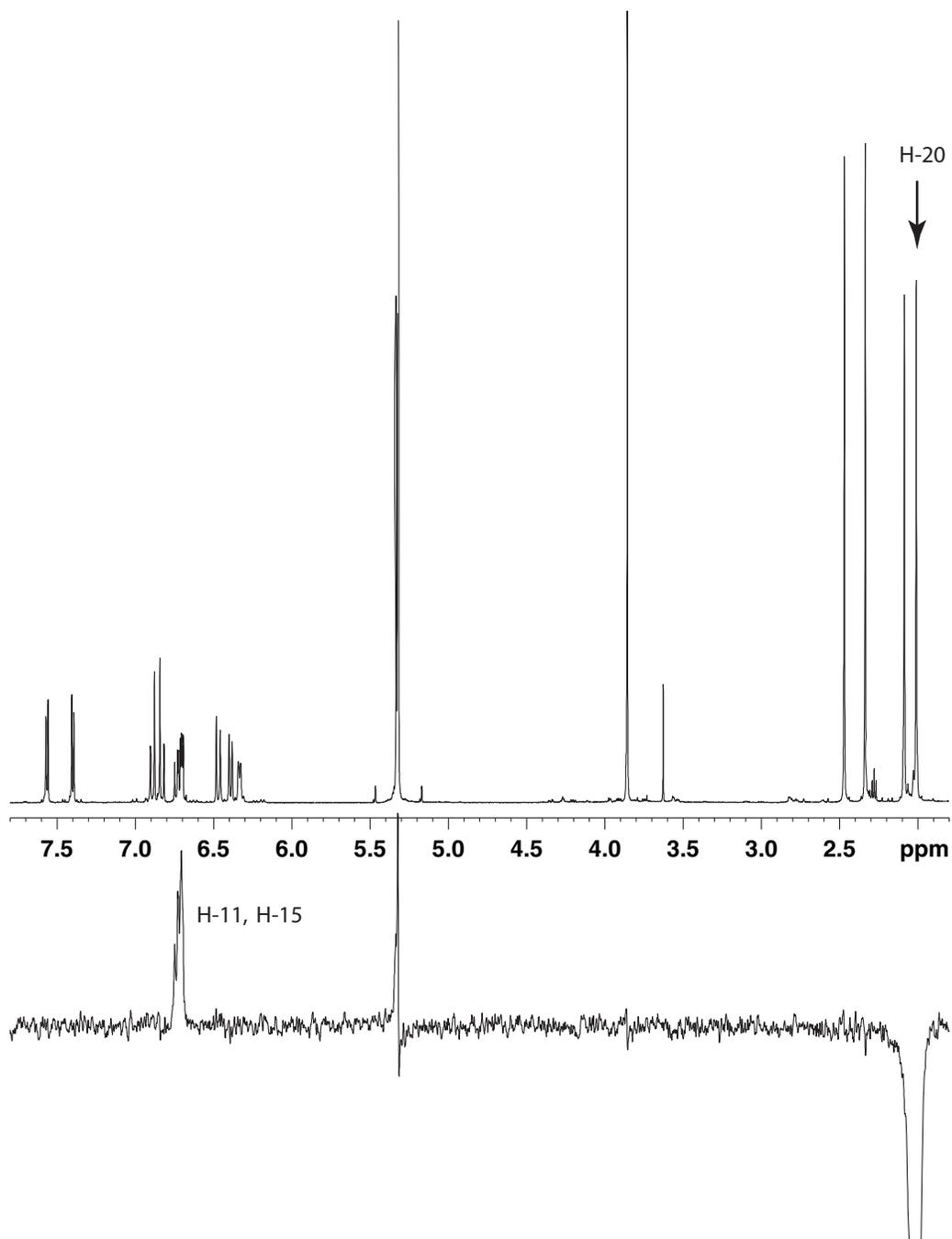


Figure S11: 1D-ROE difference spectrum of synechoxanthin dimethyl ester in  $\text{CD}_2\text{Cl}_2$ . Top trace, reference spectrum; bottom trace, difference spectrum obtained after inversion of  $\text{C}_{20}\text{H}_3$  and locking.

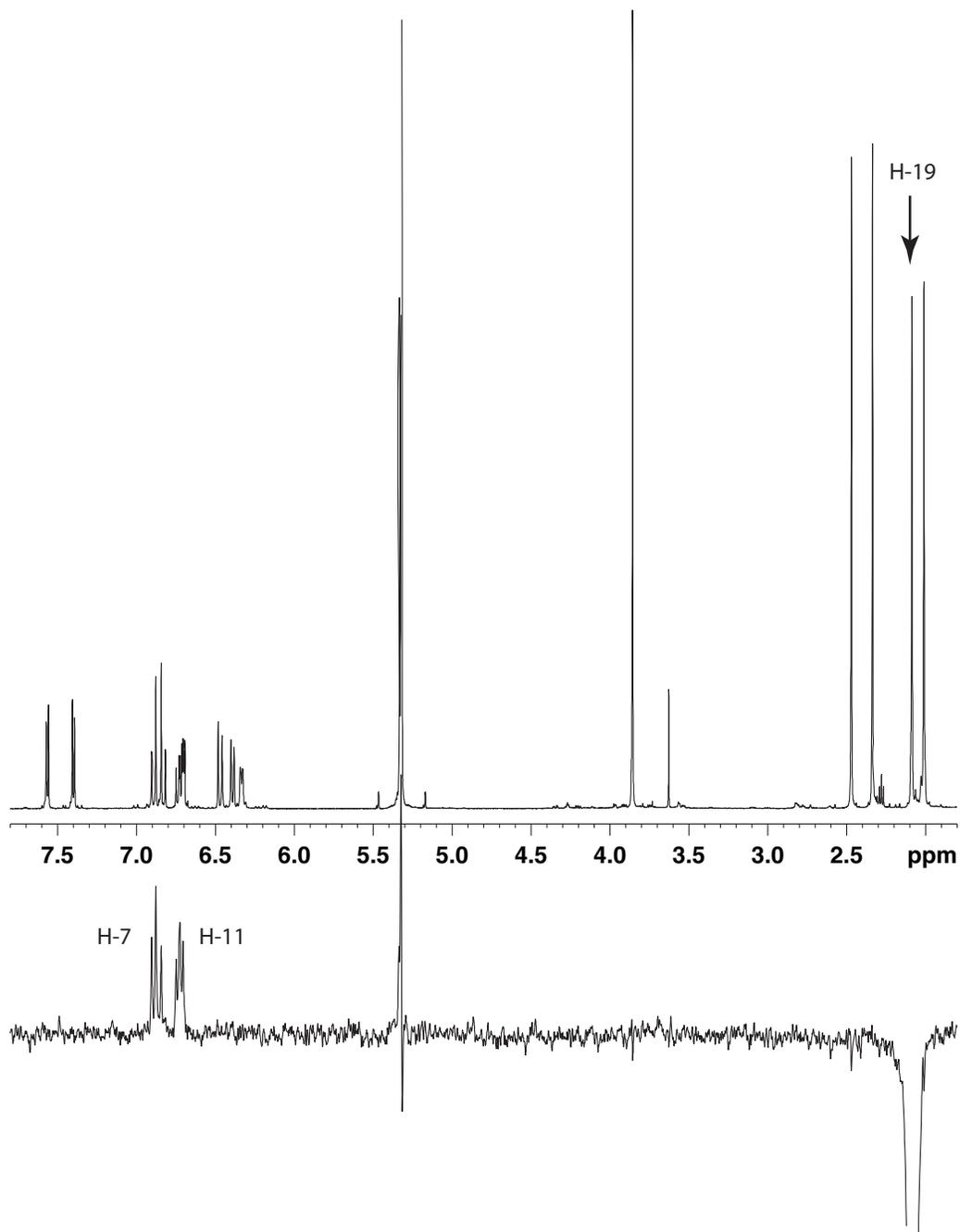


Figure S12: 1D-ROE difference spectrum of synechoxanthin dimethyl ester in CD<sub>2</sub>Cl<sub>2</sub>. Top trace, reference spectrum; bottom trace, difference spectrum obtained after inversion of C<sub>19</sub>H<sub>3</sub> and locking.

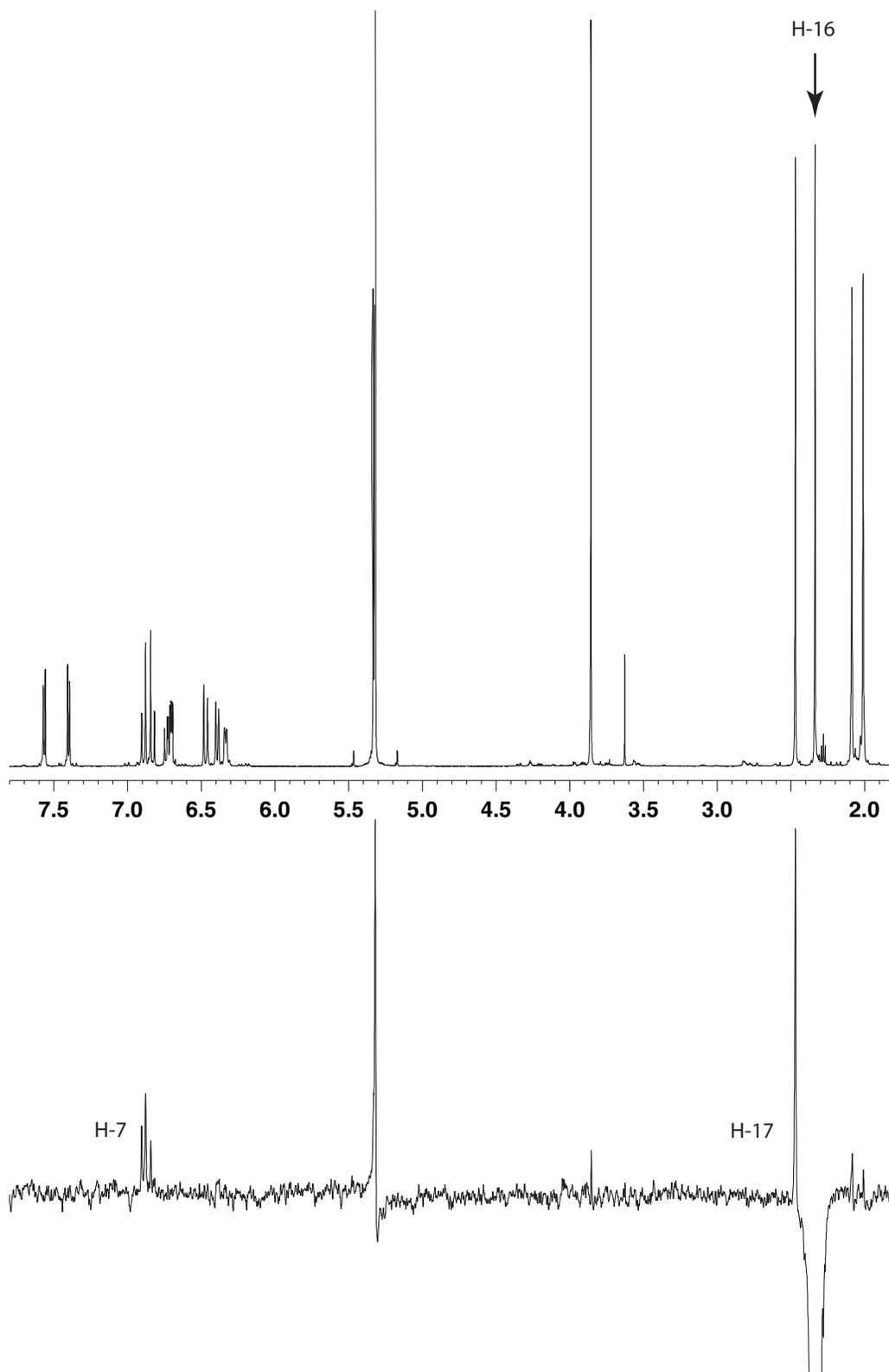


Figure S13: 1D-ROE difference spectrum of synechocanthin dimethyl ester in  $\text{CD}_2\text{Cl}_2$ . Top trace, reference spectrum; bottom trace, difference spectrum obtained after inversion of  $\text{C16H}_3$  and locking.

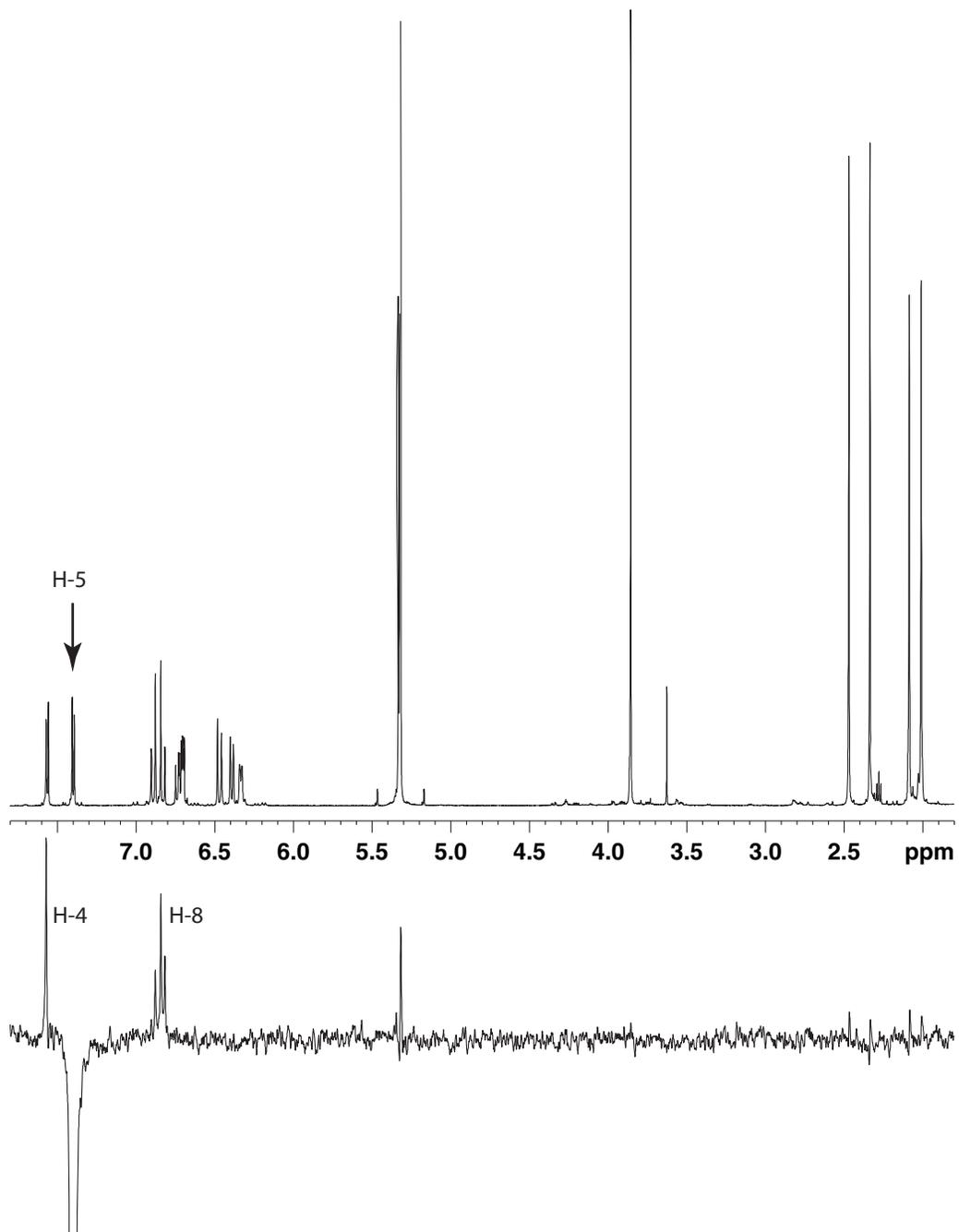


Figure S14: 1D-ROE difference spectrum of synechoxanthin dimethyl ester in  $\text{CD}_2\text{Cl}_2$ . Top trace, reference spectrum; bottom trace, difference spectrum obtained after inversion of H-5 and locking.

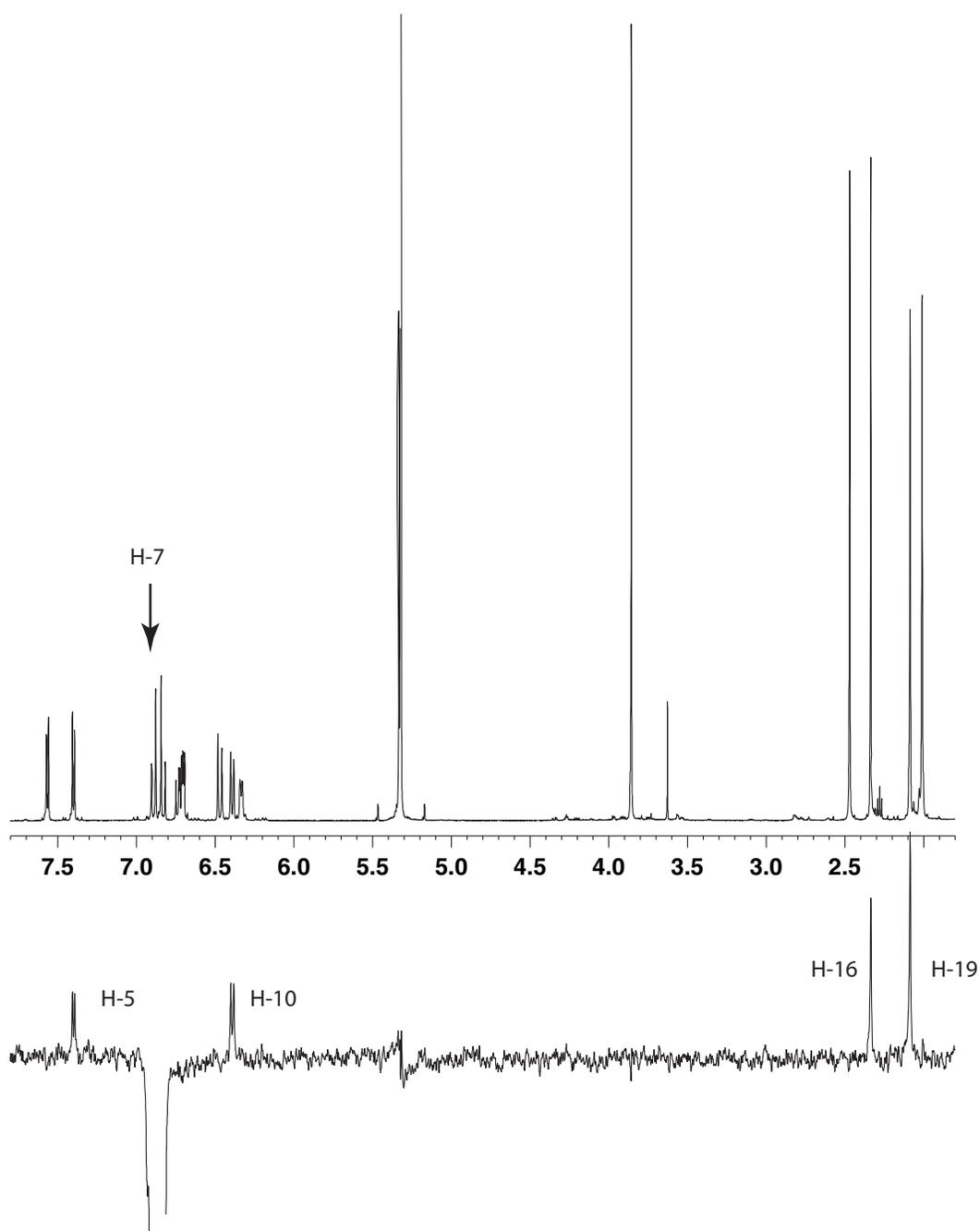


Figure S15: 1D-ROE difference spectrum of synechococcal dimethyl ester in  $\text{CD}_2\text{Cl}_2$ . Top trace, reference spectrum; bottom trace, difference spectrum obtained after inversion of H-7 and locking. H-8 was also perturbed.

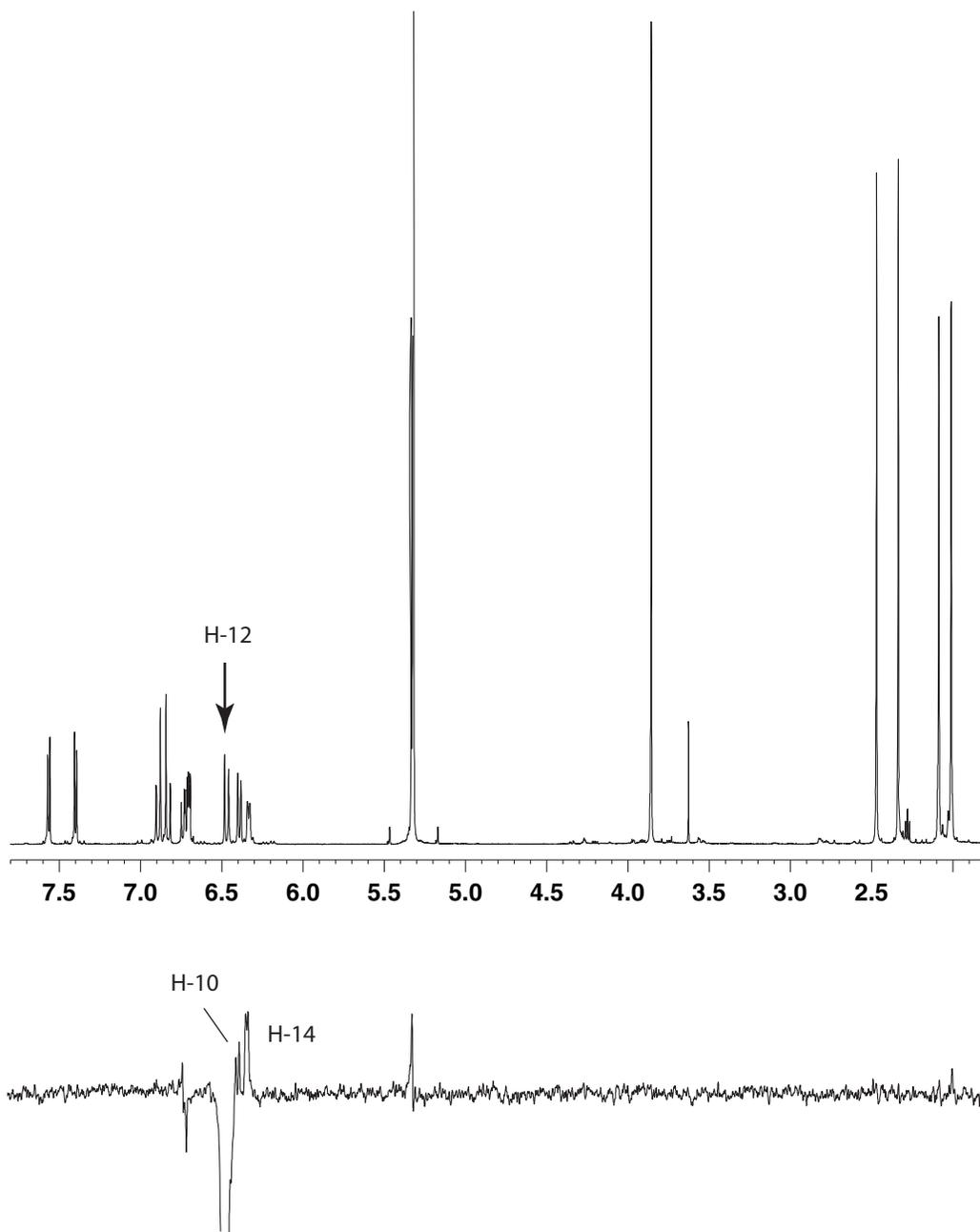


Figure S16: 1D-ROE difference spectrum of synechoxanthin dimethyl ester in  $\text{CD}_2\text{Cl}_2$ . Top trace, reference spectrum; bottom trace, difference spectrum obtained after inversion of H-12 and locking.

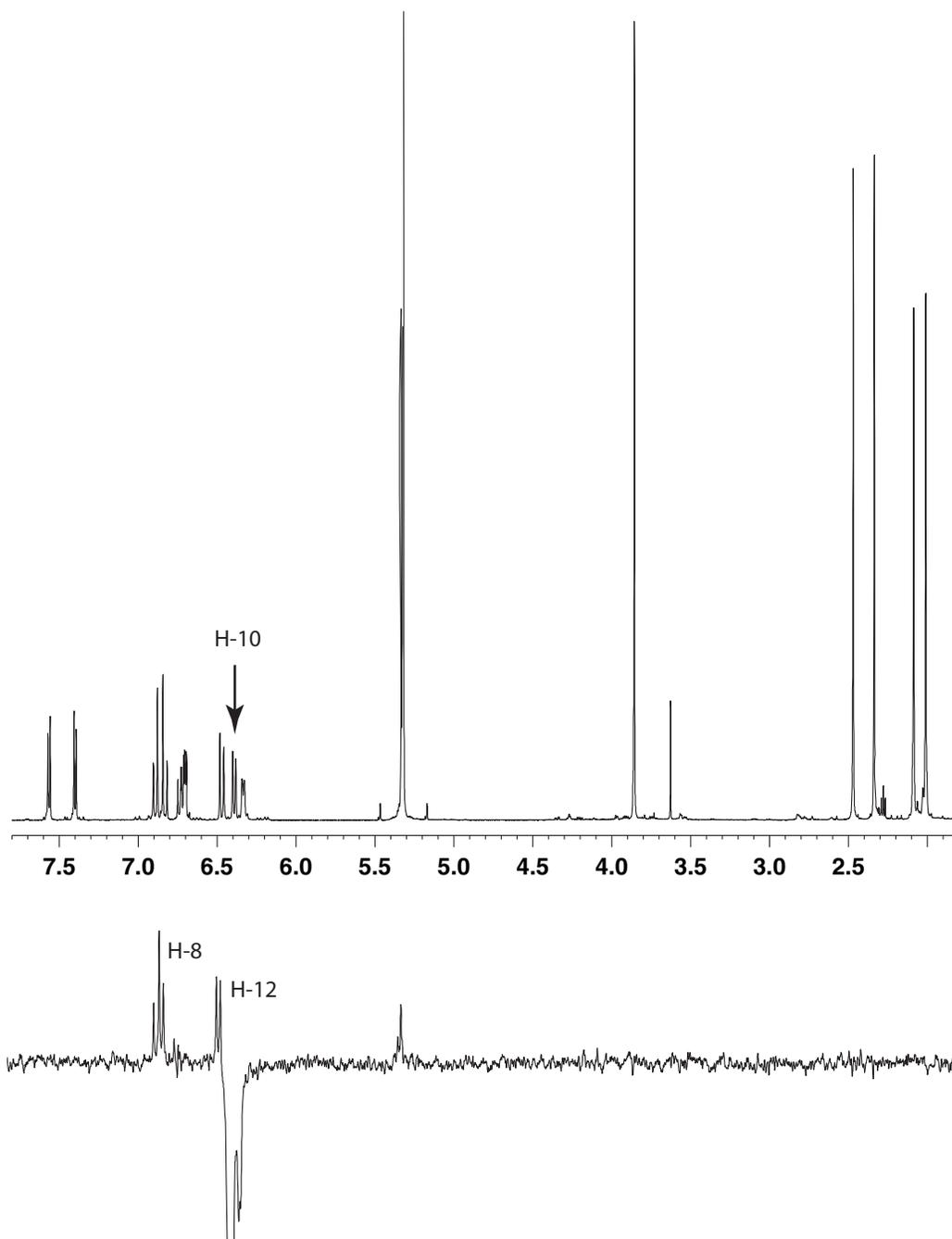


Figure S17: 1D-ROE difference spectrum of synechocanthin dimethyl ester in  $\text{CD}_2\text{Cl}_2$ . Top trace, reference spectrum; bottom trace, difference spectrum obtained after inversion of H-10.

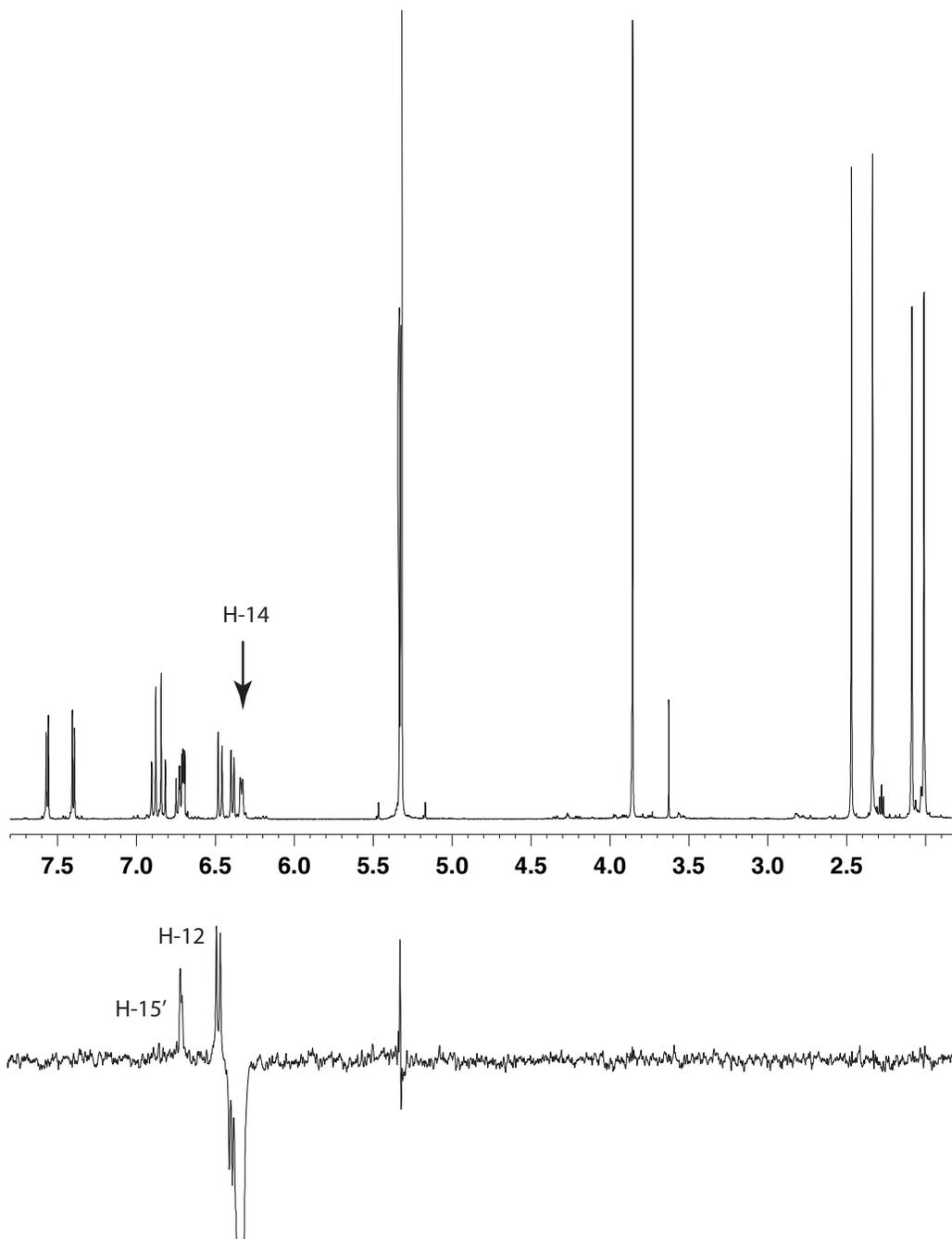
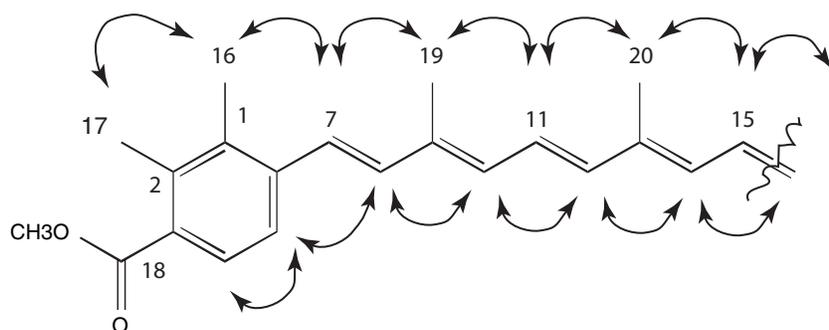


Figure S18: 1D-ROE difference spectrum of synechoxanthin dimethyl ester in  $\text{CD}_2\text{Cl}_2$ . Top trace, reference spectrum; bottom trace, difference spectrum obtained after inversion of H-14.



Scheme S4: 1D-ROE connectivities. These are in agreement with the structure. Note that it was not possible to be completely selective in inverting H-7 and H-8.

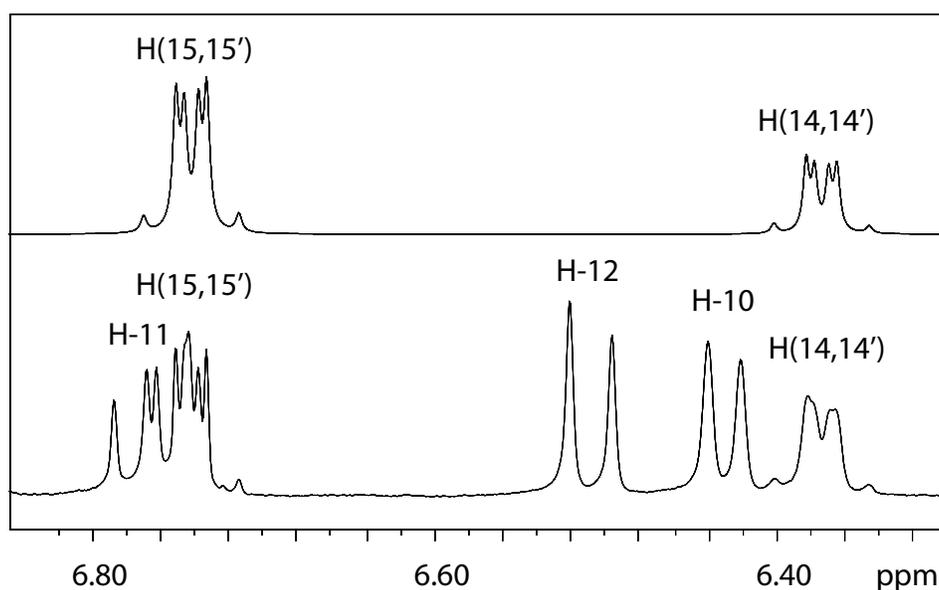


Figure S19: Top trace: Simulation of the AA'BB' spin system of H(14,14') and H(15,15'). The parameters were  $J_{14,15} = J_{14',15'} = 11.9$  Hz;  $J_{15,15'} = 14.3$  Hz;  $J_{14,15'} = J_{15,14'} = -1$  Hz, in agreement with spectra of polyenes with *trans* configuration at the 15–15' bond (Englert, G. In Carotenoids; Britton, G.; Liaaen-Jensen, S.; Pfander, H., Eds.; Birkhäuser: Basel, 1995; Vol. 1B Chapter 6). Bottom trace: Experimental spectrum, which contains the resonances of H-11, H-12 and H-10 (see Figure 4) in the same region.

Table S1: Benzene ring  $^{13}\text{C}$  shifts (ppm)

	$\delta_{\text{obs}}$	$\delta_{\text{calc}}$	$\Delta\delta$
C-1	135.6	136.0	0.4
C-2	138.0	139.9	1.9
C-3	130.0	130.6	0.6
C-4	127.3	126.9	-0.4
C-5	122.7	123.0	0.3
C-6	140.2	143.0	2.8

$\delta_{\text{obs}}$ : measured chemical shift;  $\delta_{\text{calc}}$ : calculated value based on benzene substituent effects;  $\Delta\delta = \delta_{\text{calc}} - \delta_{\text{obs}}$ . Values were calculated with a base shift of 128.5 ppm and the contributions listed in Silverstein & Webster, 6<sup>th</sup> Ed. p 229.

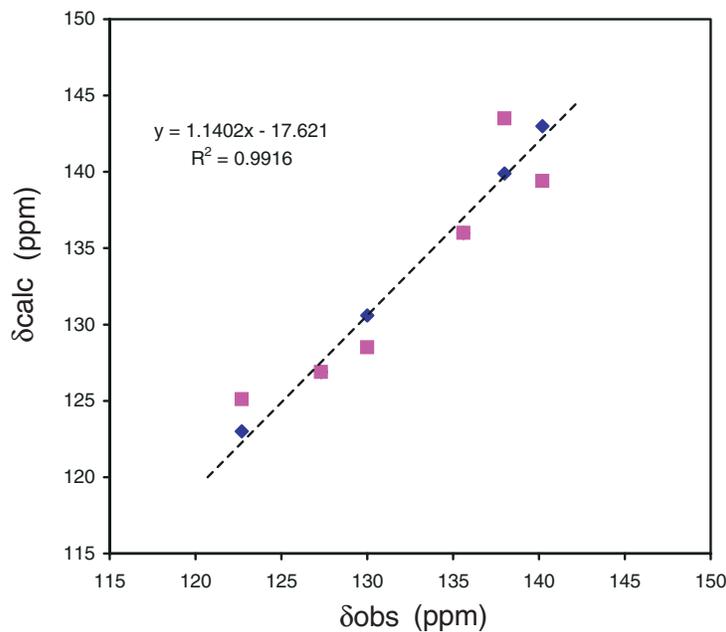


Figure S20: Correlation between observed and calculated  $^{13}\text{C}$  chemical shifts for the benzene ring. The blue symbols and the regression are for synechoxanthin dimethyl ester ( $\chi$  substitution); the pink symbols are for a compound with the methyl ester at position 5 ( $\phi$  substitution).