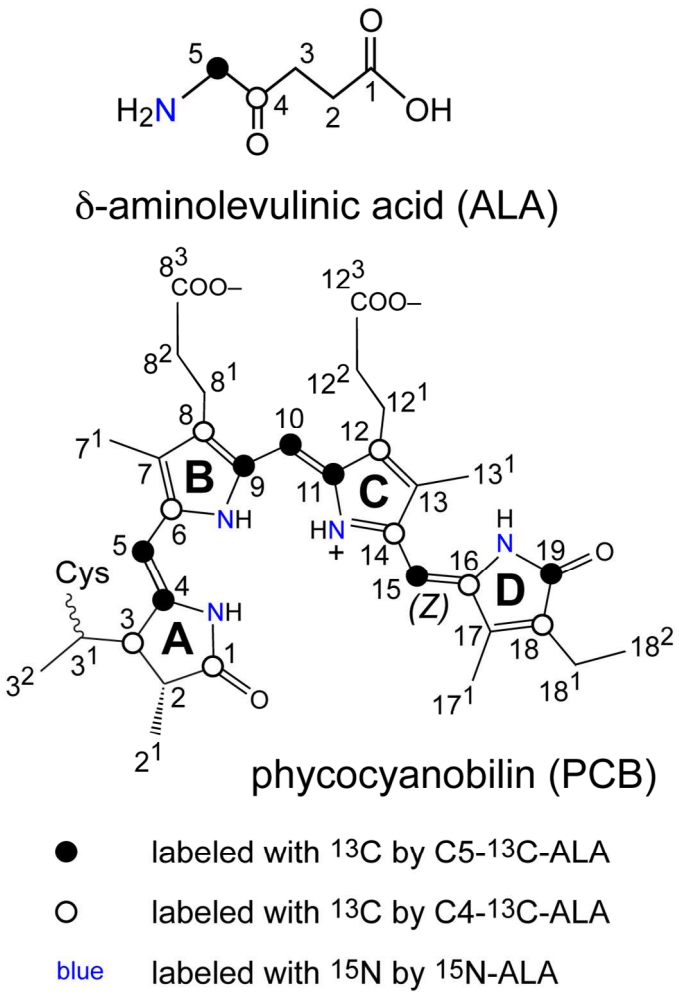

Figure S1. Labeling and numbering schemes for the PCB chromophore of NpR6012g4. The numbering scheme is shown for the bilin precursor δ-aminolevulinic acid (*top*) and for the PCB adduct of NpR6012g4 (*bottom*). PCB atoms labeled by C5-^{13}C-ALA (C5-ALA, filled circles), C4-^{13}C-ALA (C4-ALA, empty circles), and ^{15}N-ALA (blue) are indicated.

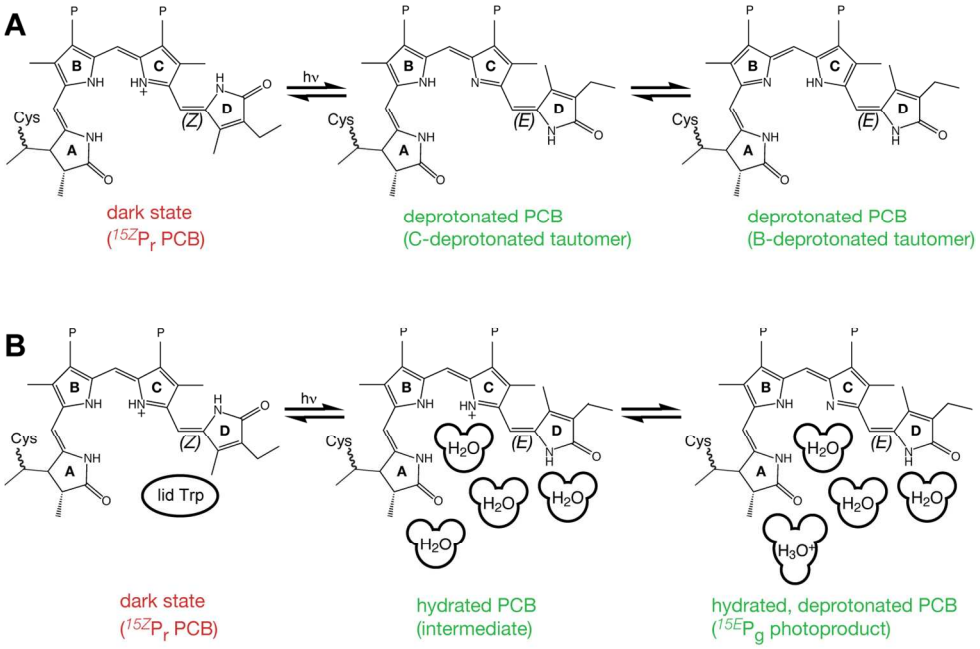

Figure S2. Alternate models for the red/green photocycle. (A) The protochromic model. (B) The hydration/solvation model.

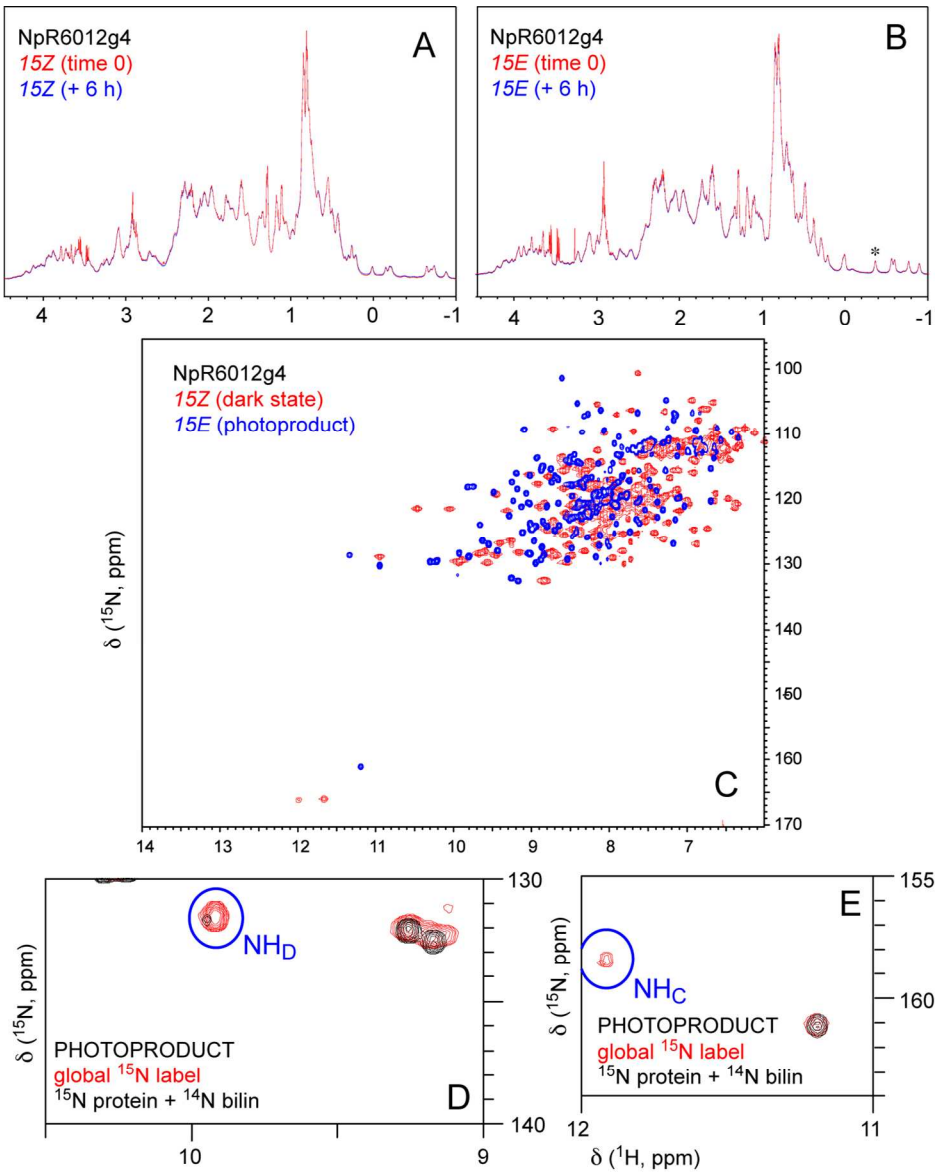

Figure S3. Initial characterization of NpR6012g4 by NMR spectroscopy. (A) 1D spectra of the NpR6012g4 dark state before (red) and after (blue) 6 h of data acquisition. (B) 1D spectra of the NpR6012g4 photoproduct before (red) and after (blue) 6 h of data acquisition. The asterisk at –0.4 ppm indicates diagnostic peak for photoproduct. The 1D spectra were acquired with 16 scans, recycle delay of 3 seconds, and acquisition time of 1.5 seconds. The ^{1}H spectral sweep width was set to 15 ppm with a ^{1}H carrier frequency of 4.70 ppm. (C) ^{1}H–^{15}N HSQC spectra of the NpR6012g4 dark state (red) and photoproduct (blue). NpR6012g4 protein was labeled with ^{15}N, and PCB chromophore was labeled with C5-^{13}C ALA (Table S1). The ^{15}N (F1) and ^{1}H (F2) carrier frequencies were 140 and 4.70 ppm. Acquisition times were 14 ms (F1) and 200 ms (F2). (D) An expanded view of the region of the HQSC spectrum in the vicinity of the D-ring NH cross-peak is shown. Spectra from one sample labeled with both C5-^{13}C ALA (natural

abundance ^{14}N) and $^{15}NH_4^+$ (black) and another labeled with $^{15}NH_4^+$ only (red) are overlaid. (E) A similar view is shown in the vicinity of the C-ring NH cross-peak.

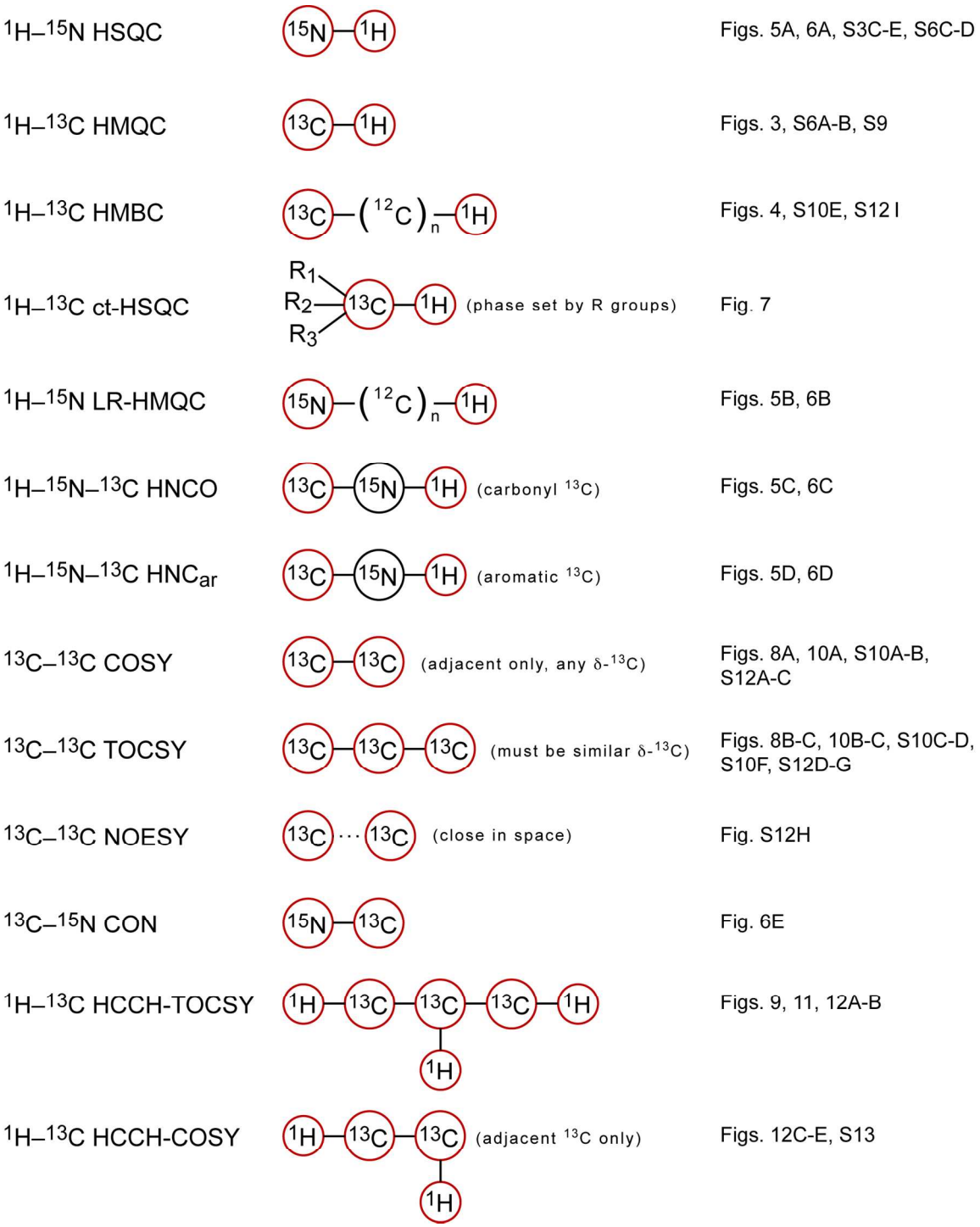

Figure S4. Pulse sequences used in this work. Atoms that must be NMR active for a given pulse sequence are circled. Atoms that can be detected in 2D or 3D spectra are circled in red.

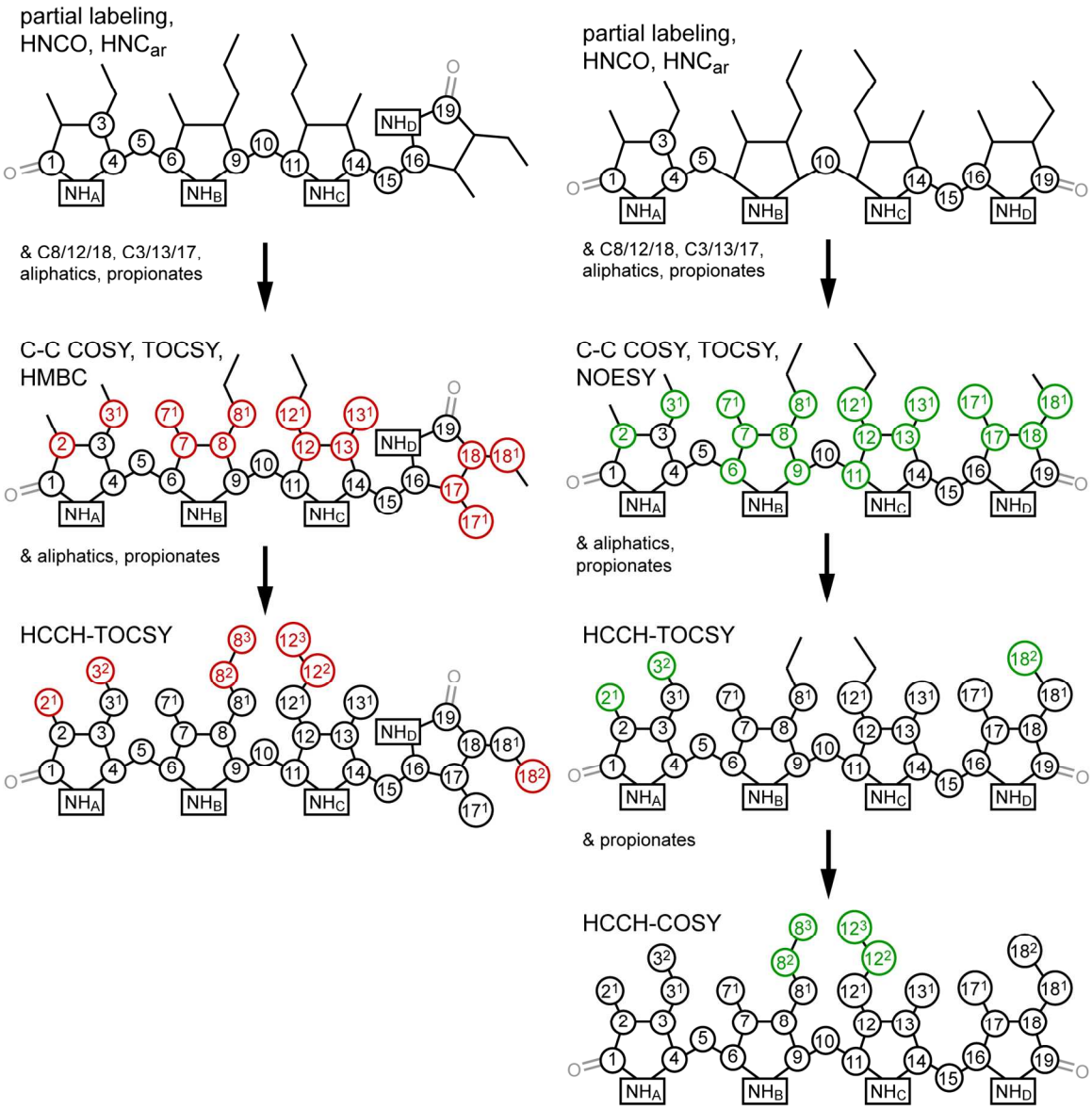

Figure S5. Assignment scheme for ^{13}C and ^{15}N resonances in NpR6012g4. Atoms identified at each step are highlighted. The process is shown for the dark state (*left*) and photoproduct (*right*).

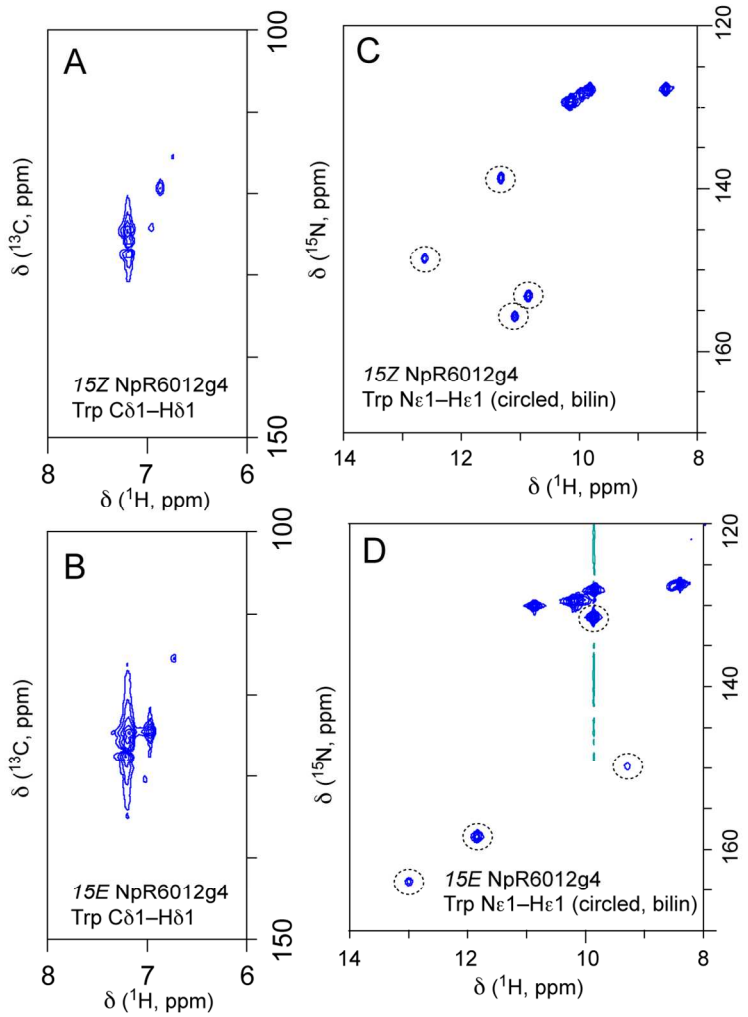

Figure S6. Trp resonances of NpR6012g4. (A,B) NpR6012g4 was labeled with ^{15}N-ALA and 2-^{13}C-indole (Table S1) and characterized using ^{1}H–^{13}C HMQC spectroscopy. Spectra are shown for the dark state (A) and photoproduct (B). The ^{13}C (F1) and ^{1}H (F2) carrier frequencies were 80 ppm and 4.70 ppm. The acquisition times were 10.6 ms (F1) and 228 ms (F2). (C-D) NpR6012g4 labeled with both *u*-^{13}C, ^{15}N-ALA and ^{15}N-indole and characterized using ^{1}H–^{15}N HSQC. Spectra are shown for the dark state (C) and photoproduct (D). Bilin resonances are circled in the HSQC spectra, for which the ^{15}N (F1) and ^{1}H (F2) carrier frequencies were 140 and 4.70 ppm and the acquisition times were 14 ms (F1) and 200 ms (F2).

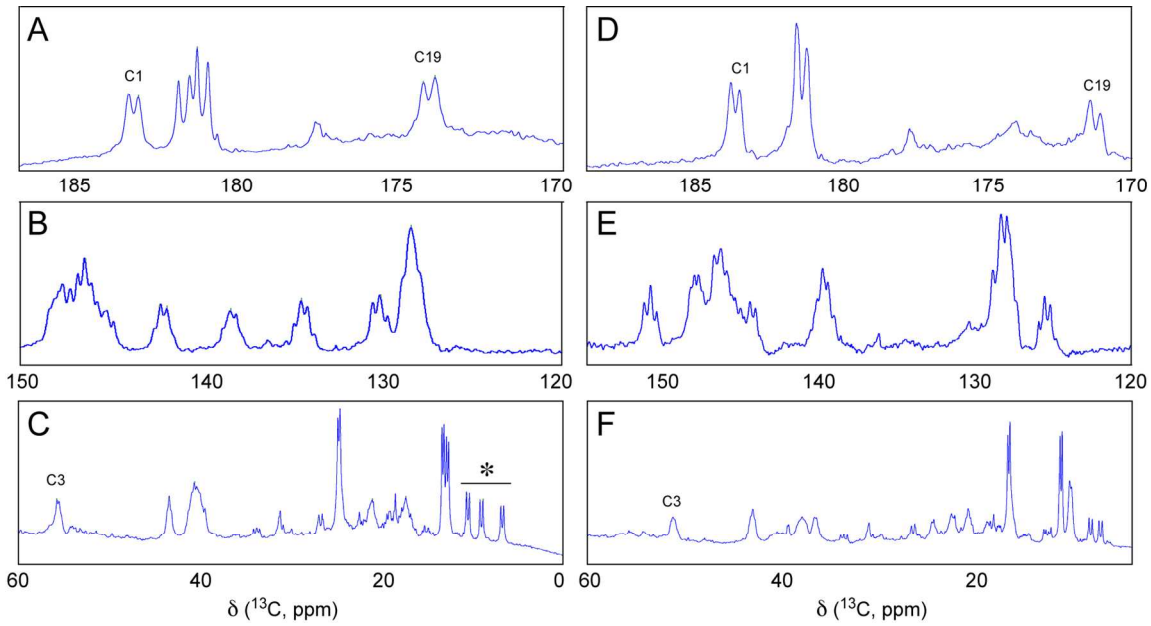

Figure S7. One-dimensional ^{13}C NMR spectra of globally labeled NpR6012g4. Detailed regions are shown for the dark state (A-C) and photoproduct (D-F). One-dimensional ^{13}C NMR spectra (with ^{1}H decoupling by WALTZ-16 applied during the acquisition) were acquired with 2048 scans, recycle delay of 2 seconds, ^{13}C and ^{1}H carrier frequencies of 100 ppm and 4.70 ppm, and acquisition time of 1.1 seconds.

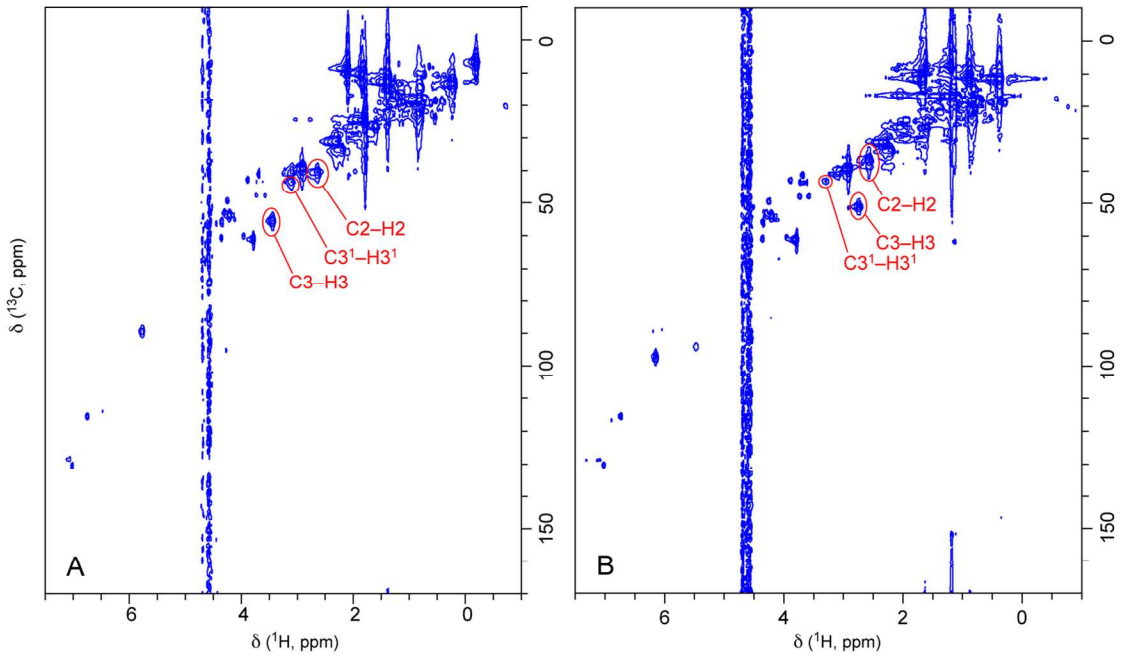

Figure S8. Characterization of globally labeled NpR6012g4 using ^{1}H–^{13}C HMQC spectroscopy. Spectra are shown for the dark state (A) and photoproduct (B). Resonances from the tertiary aliphatic carbons of the A-ring are indicated. The ^{13}C (F1) and ^{1}H (F2) carrier frequencies were 80 and 4.70 ppm. The acquisition times were 10 ms (F1) and 228 ms (F2).

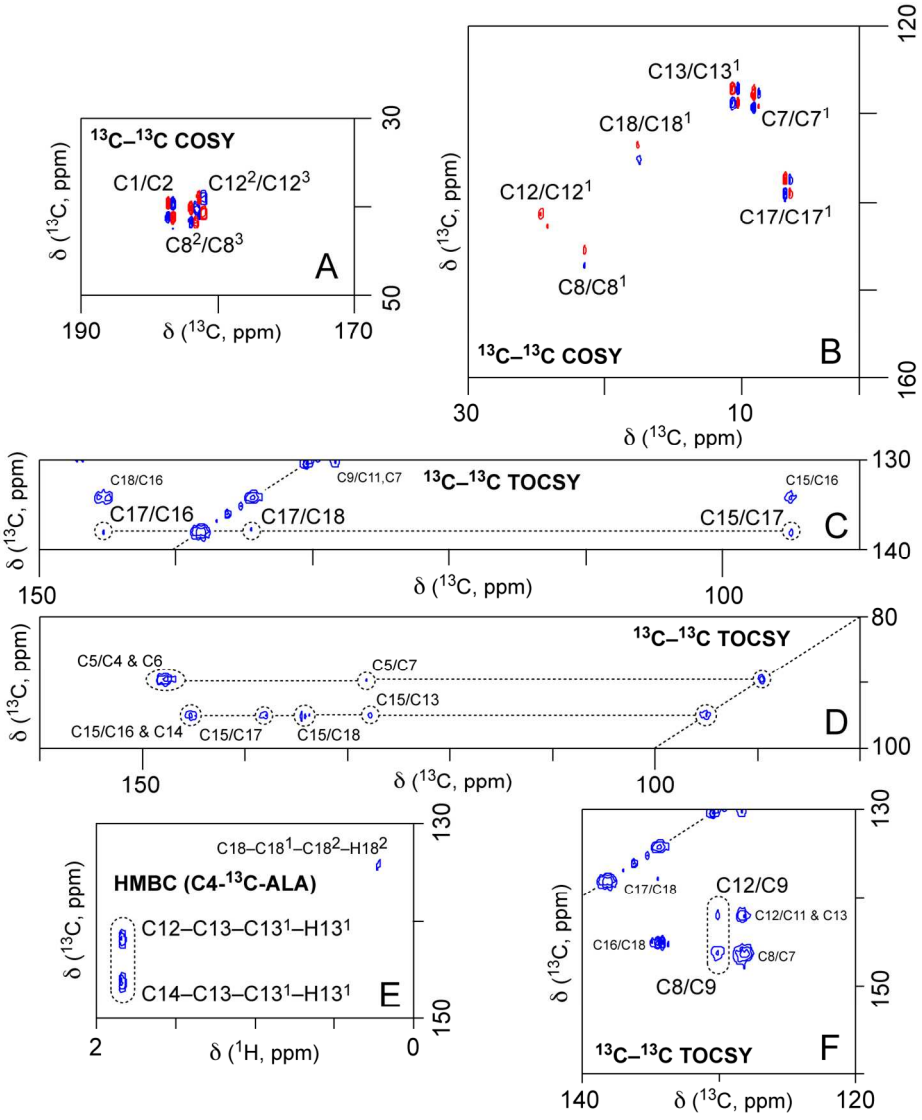

Figure S9. Assignment of ring carbons for the red-absorbing NpR6012g4 dark state. (A) A detail view is shown for carbonyl/aliphatic cross-peaks in the ^{13}C–^{13}C COSY spectrum (Fig. 8A), with assignments indicated. (B) A detail view is shown of aromatic/aliphatic cross-peaks in the ^{13}C–^{13}C COSY spectrum. (C) A detail view of the C7 strip in the ^{13}C–^{13}C TOCSY spectrum is shown. (D) A detail view of the C5 and C15 strips in the ^{13}C–^{13}C TOCSY spectrum is shown. (E) A detail view is shown for the HMBC spectrum of NpR6012g4 labeled at C1, C3, C6, C8, C12, C14, C16, and C18 (Fig. 4A). Assignments are indicated. The ^{13}C (F1) and ^{1}H (F2) carrier frequencies were 120 ppm and 4.70 ppm. The acquisition times were 10.6 ms (F1) and 228 ms (F2). (F) A detail view of C8 and C12 resonances in the ^{13}C–^{13}C TOCSY spectrum is shown.

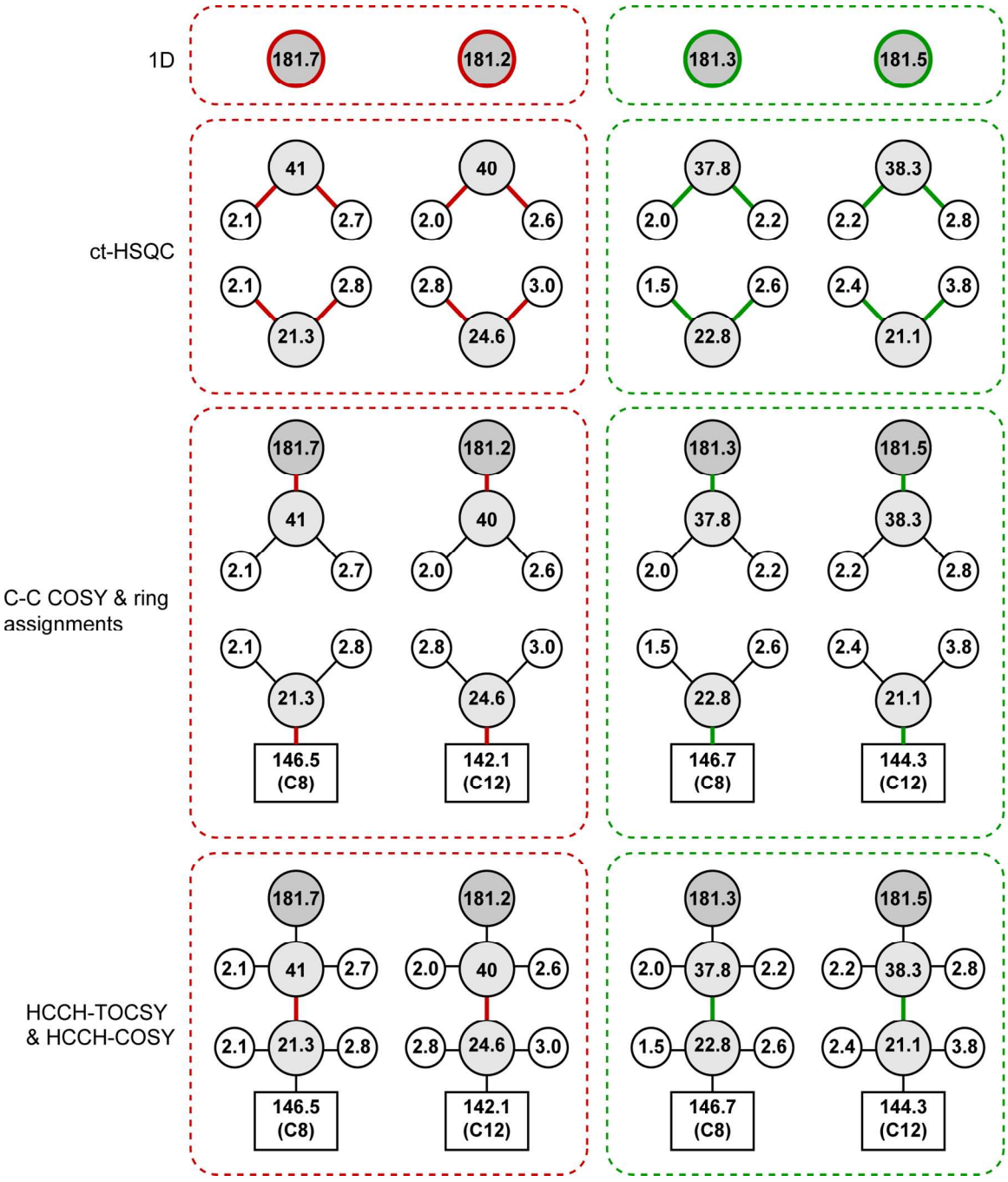

Figure S10. Assignment strategy for propionate side chains. Connectivity established at each step is highlighted in color. Dark state (boxed in red) and photoproduct (boxed in green) are depicted.

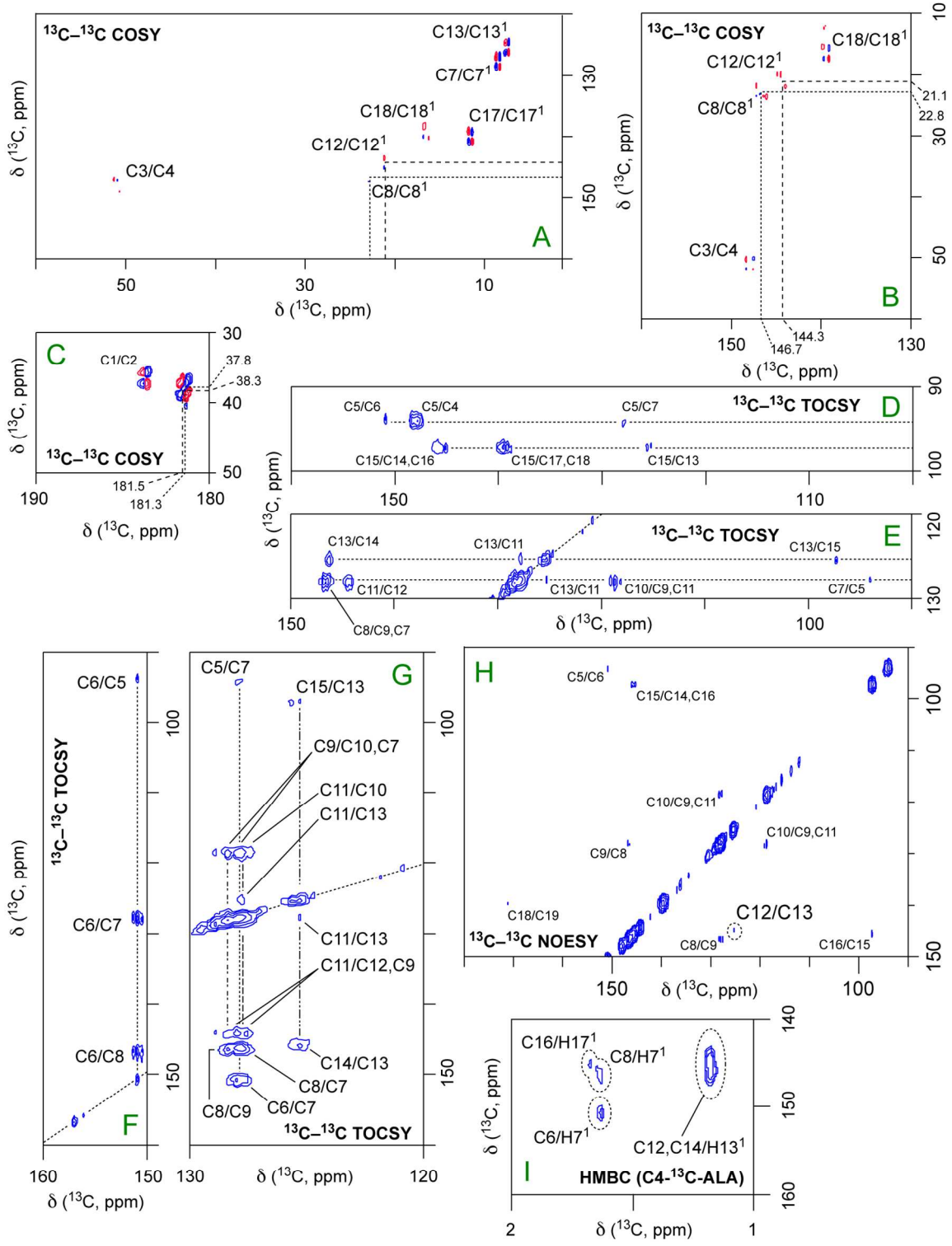

Figure S11. Assignment of ring carbons in the green-absorbing NpR6012g4 photoproduct state. (A) A detail view is shown of aromatic/aliphatic cross-peaks in the ^{13}C–^{13}C COSY spectrum (Fig. 10A). (B) A detail view is shown for the mirror region matching panel A. (C) A detail view is shown of carbonyl/aliphatic cross-peaks in the

^{13}C–^{13}C COSY spectrum. Known chemical shifts for the β-methylene carbons of the propionate side chains (Fig. 7B) permitted resolution of the γ-carbonyl atoms. (D) A detail view of the C5 and C15 strips in the ^{13}C–^{13}C TOCSY spectrum is shown. (E) A detail view of the C7, C11, and C13 strips in the ^{13}C–^{13}C TOCSY spectrum is shown. (F) A detail view of the C6 strip in the ^{13}C–^{13}C TOCSY spectrum is shown. (G) A detail view of additional strips in the ^{13}C–^{13}C TOCSY spectrum is shown. Spectral parameters for panels A-G are described in Fig. 10. (H) The aromatic region of the photoproduct ^{13}C–^{13}C NOESY spectrum is shown, with the C12/C13 cross-peak circled and other assignments indicated. The ^{13}C (F1 and F2) and ^{1}H carrier frequencies were 100 ppm and 4.70 ppm. The two-dimensional acquisition times were 10 ms (F1) and 200 ms (F2). The NOESY mixing time was 200 ms. Two-dimensional spectra were acquired with 128 scans and recycle delay of 1.5 seconds. (I) A detail view is shown for the HMBC spectrum of NpR6012g4 labeled at C1, C3, C6, C8, C12, C14, C16, and C18 (Fig. 4B).

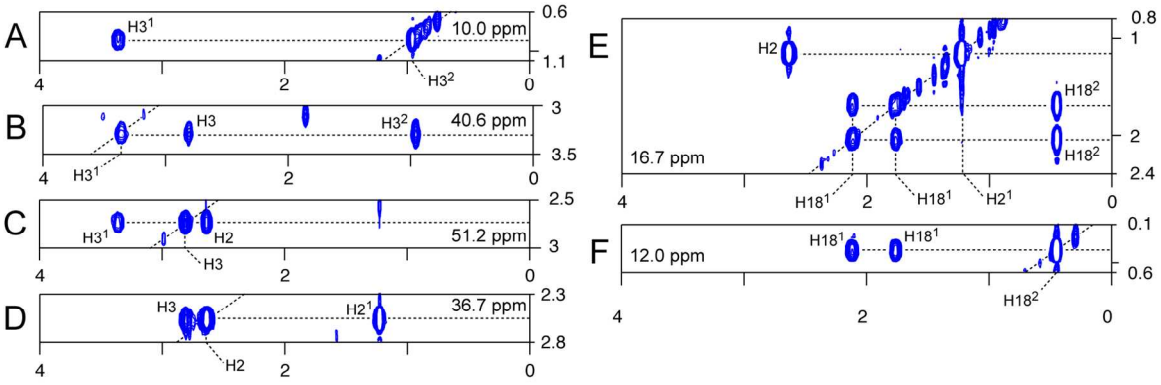

Figure S12. Confirmation of A-ring and 18-Et resonances of the green-absorbing photoproduct by HCCH-COSY. A-ring (A-E) and C18-Et (E-F) resonances are indicated. Spectra were acquired as described in Fig. 12.

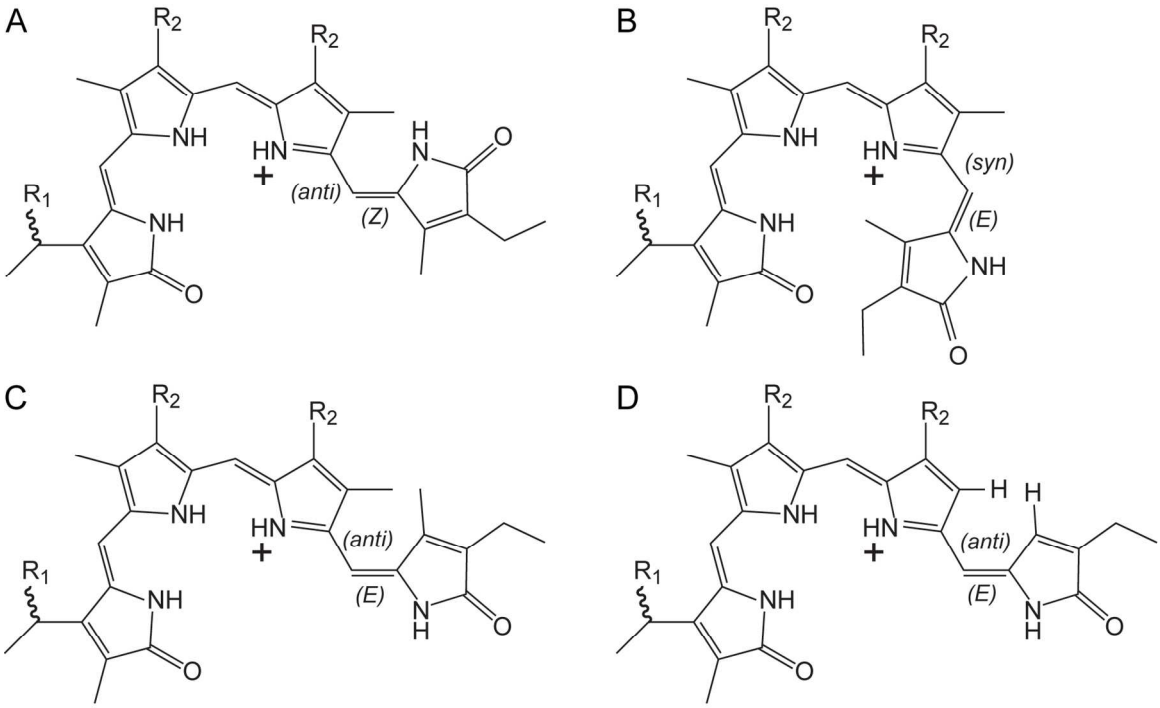

Figure S13. Model compound geometries used in *ab initio* calculations. Geometries are shown for C15-*Z,anti* (A), C15-*E,syn* (B), and C15-*E,anti* (C, D) configurations. In panel D, the 13- and 17-methyl side chains were replaced by protons in a *gedanken* experiment to assess the importance of the 13-Me/17-Me steric clash in the C15-*E,anti* configuration.

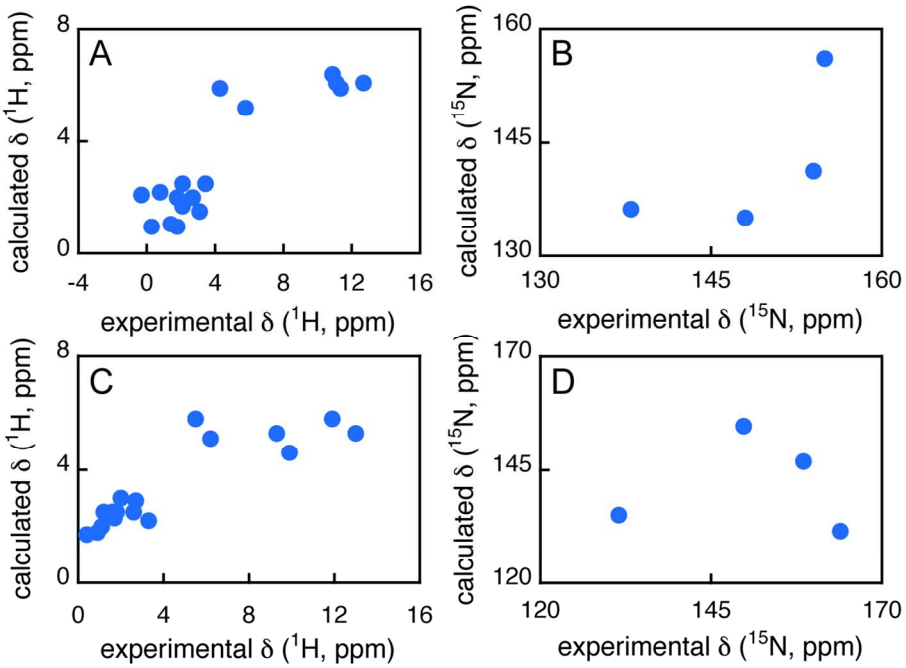

Figure S14. Comparison of calculated ^{1}H and ^{15}N chemical shifts with experimental values. (A,B) Values are plotted for the NpR6012g4 dark state. (C,D) Values are plotted for the photoproduct. (A,C) H atoms are shown. (B,D) N atoms are shown.

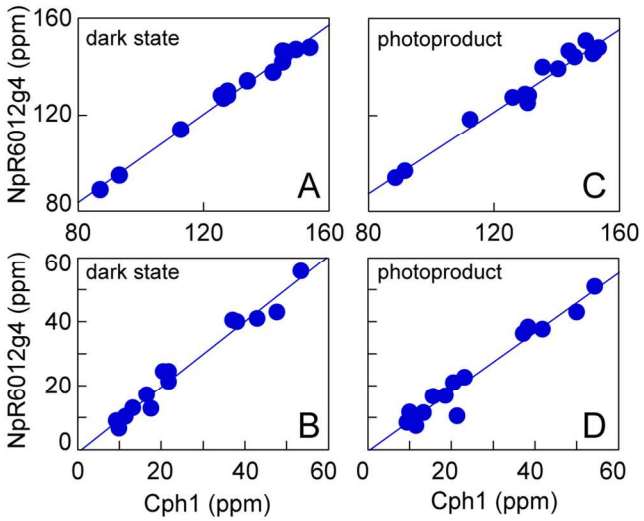

Figure S15. Comparison of ^{13}C chemical shifts in NpR6012g4 and Cph1. ^{13}C chemical shifts for NpR6012g4 are plotted versus those of Cph1 in the dark state (A-B) and photoproduct (C-D). Aromatic (A, C) and aliphatic (B, D) regions are shown.

Table S1: Labeling patterns used in this study

ALA	labeled PCB	protein precursor	protein label
–	all N	$^{15}NH_4^+$	all N
C5-ALA	C4, C5, C9, C10, C11, C15, C19	$^{15}NH_4^+$	all N
C4-ALA	C1, C3, C6, C8, C12, C14, C16, C18	$^{15}NH_4^+$	all N
^{15}N-ALA	NH_A, NH_B, NH_C, NH_D	2-^{13}C indole	Trp Cδ1
u-^{13}C,^{15}N-ALA	all C, N atoms	^{15}N indole	Trp Nε1

Table S2: C15 dihedral angles for photoproduct geometries[1]

C15 configuration	13, 17 side chains	$\chi_{14/15}$ (°)	$\chi_{15/16}$ (°)
E, syn	methyl	-51	+175
E, anti	methyl	-109	-177
E, anti	proton	-150	-176

1. $\chi_{14/15}$ is defined as the proper dihedral angle about the 14,15-bond (N_C–C14-C15-C16), and $\chi_{15/16}$ is defined as the proper dihedral angle about the 15,16-bond (N_D–C16-C15-C14). AM1 geometries were calculated as described in the Methods. Values are reported for protonated π systems.