MAS NMR study of the photoreceptor phytochrome

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Appendices

Appendix A

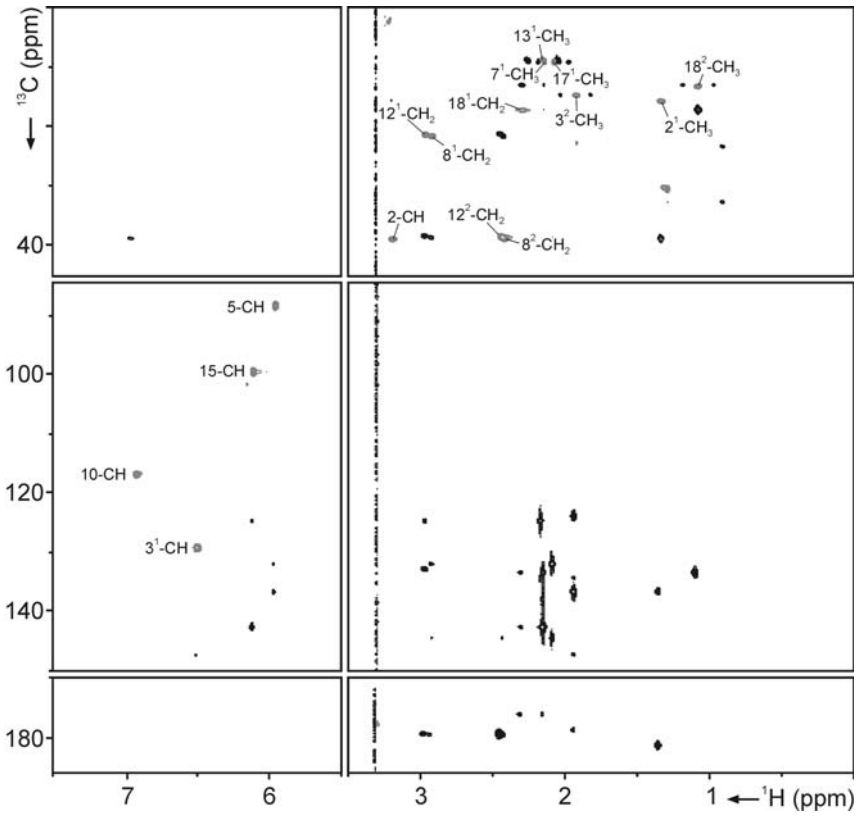

Figure A.1: Contour plot of the ^{1}H-^{13}C HSQC (black) and ^{1}H-^{13}C HMBC (grey) NMR dipolar correlation spectra of PCB in methanol-d_4 recorded in a magnetic field of 14.1 T at 277 K. The HSQC assignments of correlations are indicated.

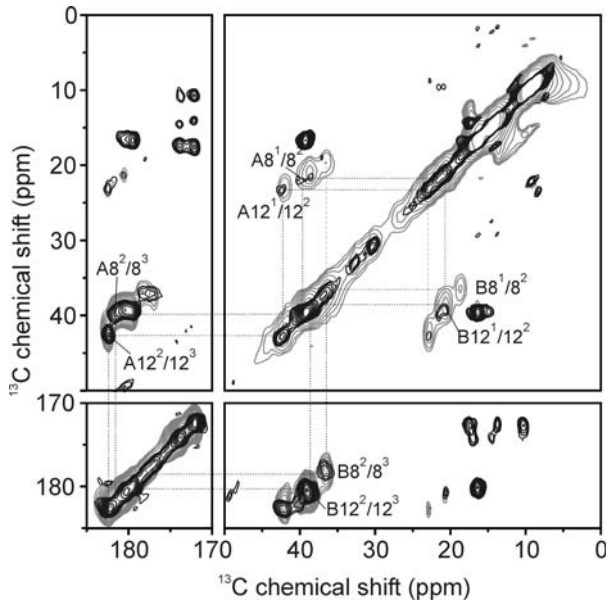

Figure A.2: Contour plot of the 2D ^{13}C-^{13}C RFDR (t_{mix} = 3.9 ms, black) and PDSD (t_{mix} = 2.4 ms, grey) MAS NMR spectra of u-[^{13}C, ^{15}N]-PCB-Cph1Δ2. The connectivity networks of the propionic acid side-chains are indicated by dotted lines.

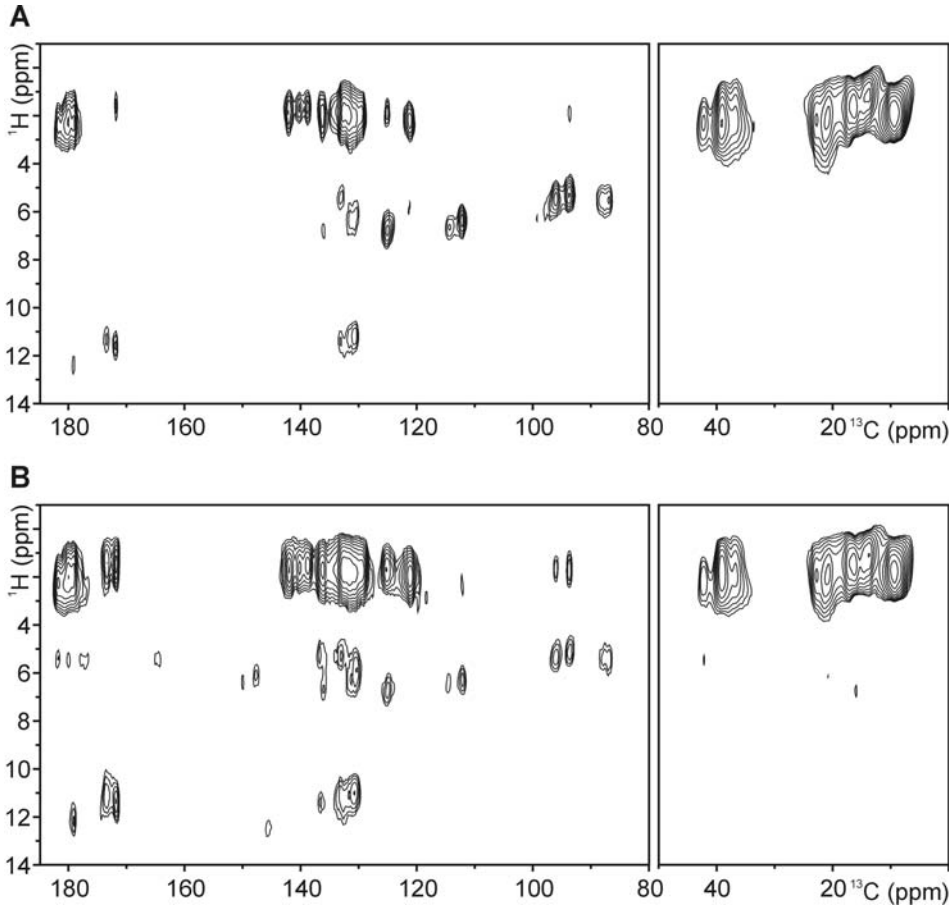

Figure A.3: Contour plot of the ^{1}H-^{13}C FSLG NMR dipolar correlation spectra of u-[^{13}C,^{15}N]-PCB recorded at 277 K in a magnetic field of 17.6 T using a spinning frequency of 12 kHz and CP contact times of 512 μs (**A**) and 1024 μs (**B**).

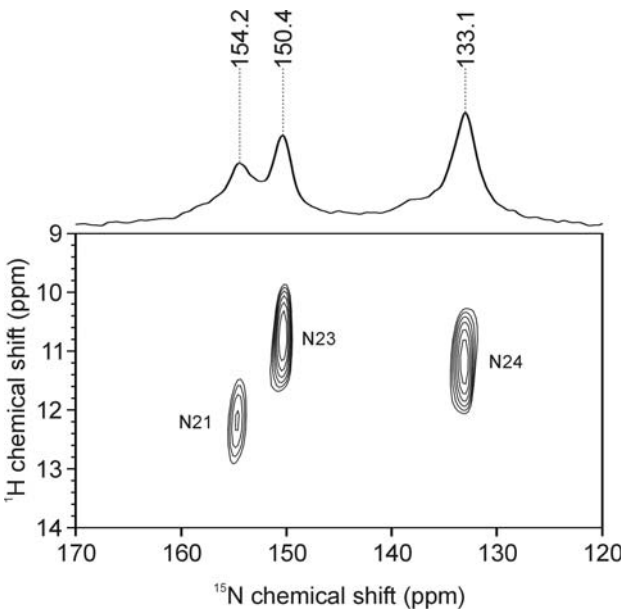

Figure A.4: Contour plots of the 2D ^{1}H-^{15}N heteronuclear dipolar correlation spectrum of u-[^{13}C, ^{15}N]-PCB recorded at 277 K in a field of 17.6 T using a spinning frequency of 7 kHz.

Appendix B

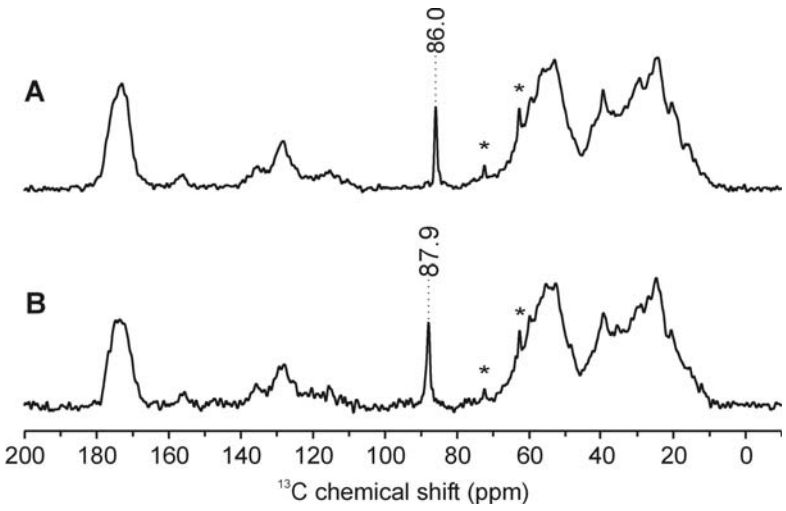

Figure B.1: 1D ^{13}C CP/MAS NMR spectra of ^{13}C5-PCB-*phyA*65 in the Pr (**A**) and Pfr (**B**) states recorded at 9.4 T, 10 kHz and 243 K. The asterisks indicate the ^{13}C response of the glycol present in the buffer.

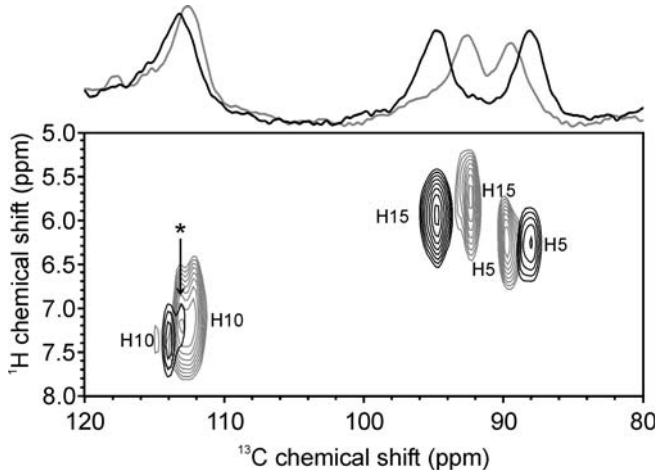

Figure B.2: Contour plot of 2D ^{1}H-^{13}C heteronuclear dipolar correlation spectra of the methine bridge region obtained from u-[^{13}C,^{15}N]-PCB-Cph1Δ2 in the the Pr (black) and Pfr (grey) states at a magnetic field of 9.4 T, 10 kHz and 243 K. The asterisk indicates the position of protein backbone signals in natural abundance.

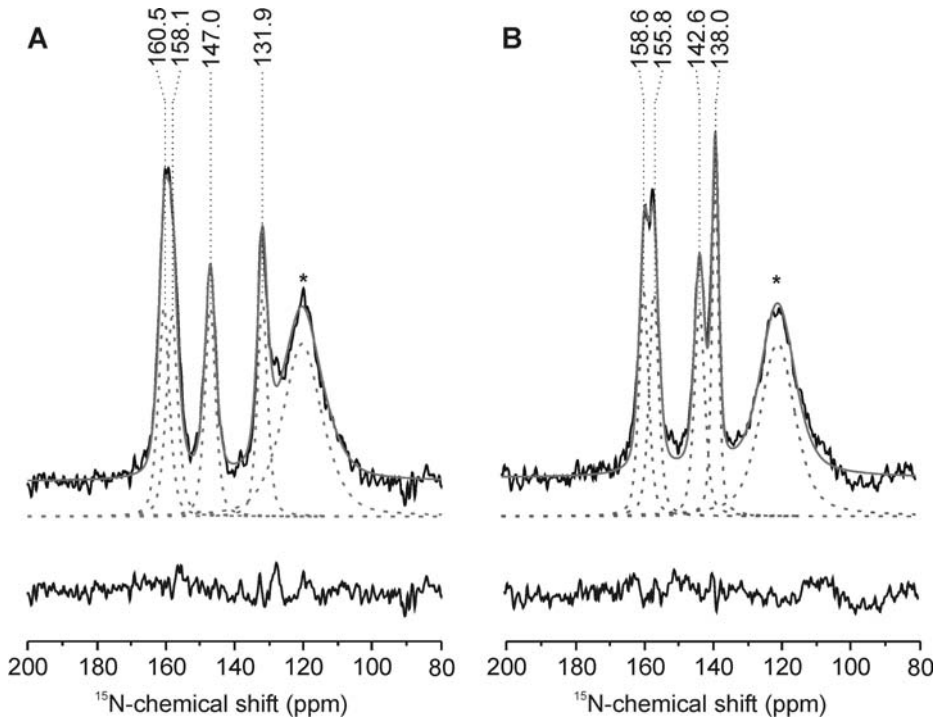

Figure B.3: Voigt deconvolution of the ^{15}N CP/MAS NMR spectra of u-[^{13}C,^{15}N]-PCB-Cph1Δ2 in the Pr (**A**, 80000 scans) and Pfr (**B**, 135000 scans) recorded in a magnetic field of 17.6 T at 243 K using a spinning frequency of 8 kHz. The upper part compares the experimental NMR spectrum with the sum of the Voigt fits. The lower part gives the individual Voigt fits (dashed lines) and the residual spectra. The asterisk indicates the ^{15}N response of the protein backbone in natural abundance.

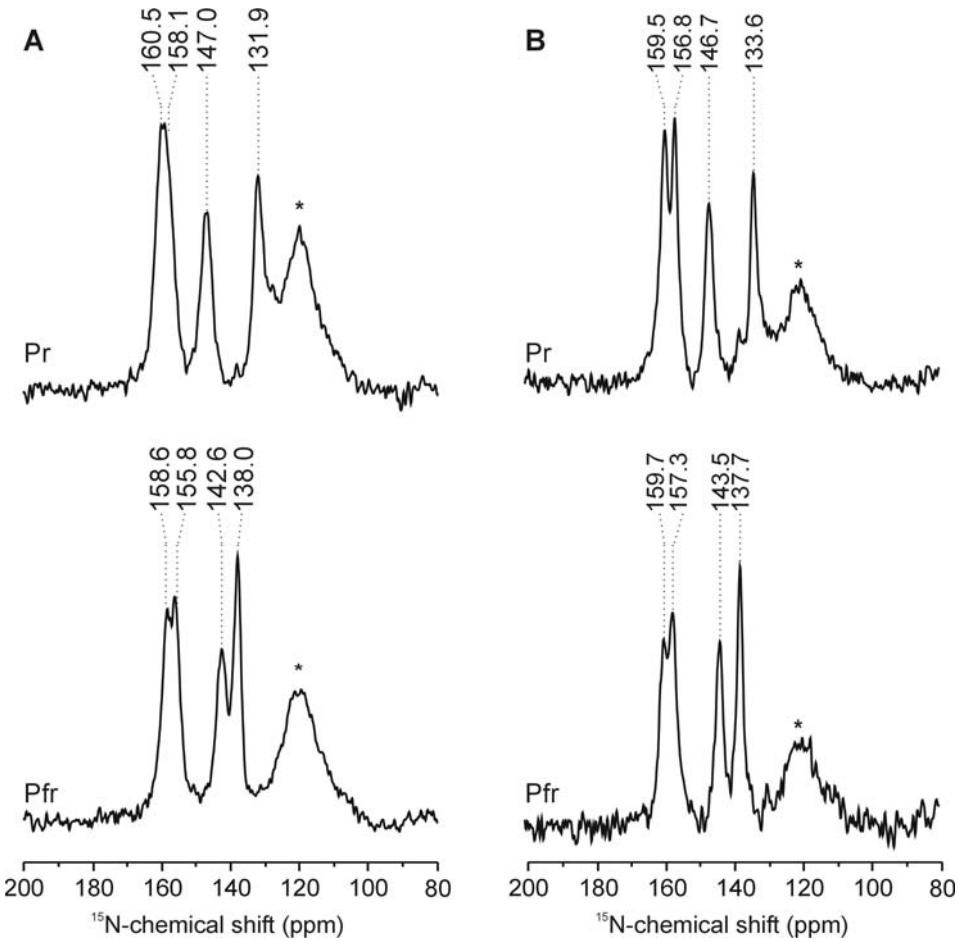

Figure B.4: ^{15}N CP/MAS NMR spectra of Cph1Δ2 (**A**) and *phyA*65 (**B**) containing an u-[^{13}C,^{15}N]-PCB in the Pr and Pfr states recorded in a magnetic field of 17.6 T at 243 K using a spinning frequency of 8 kHz. The ^{15}N response of the protein backbone in natural abundance is indicated by an asterisk.

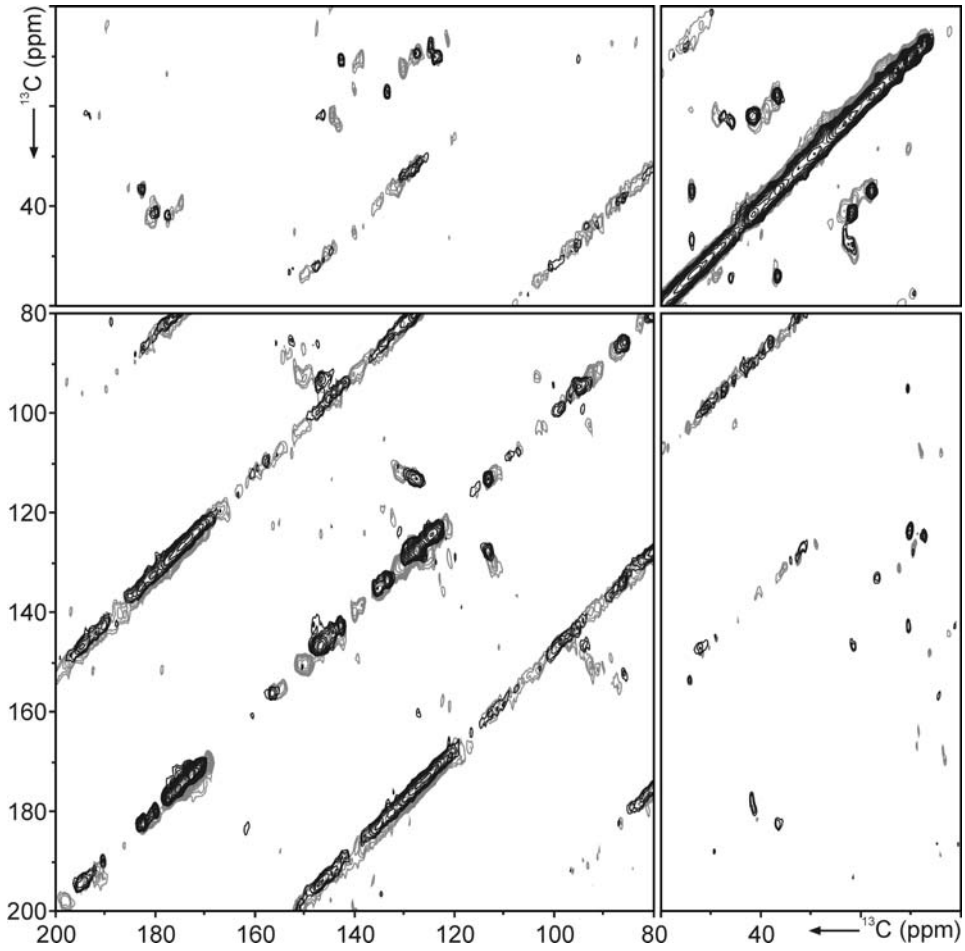

Figure B.5: Contour plot of the 2D ^{13}C-^{13}C DARR NMR spectra of u-[^{13}C,^{15}N]-PCB-*phyA*65 in the Pr (black) and Pfr states (grey). The spectra were recorded with proton mixing times of 5 ms, at 243 K and with a spinning frequency of 9 kHz.

Appendix C

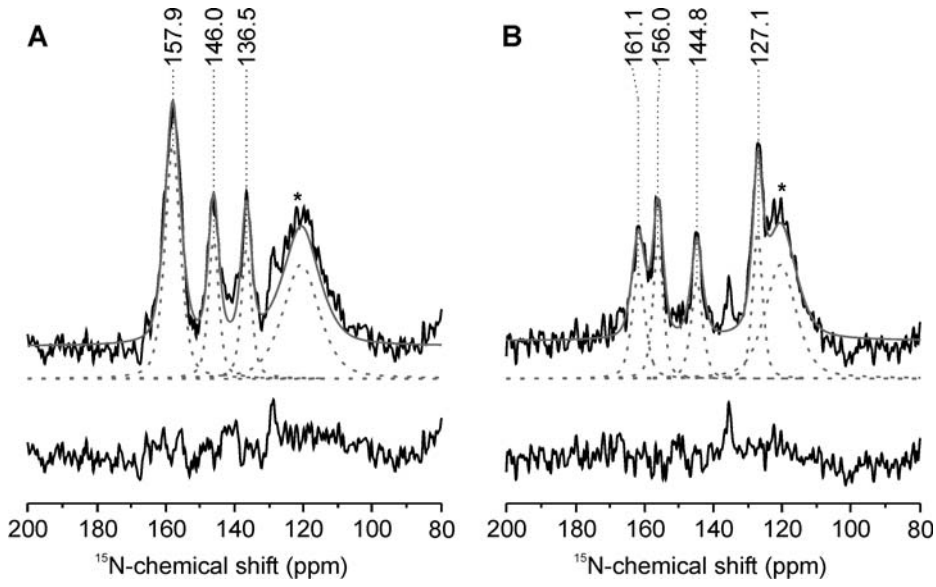

Figure C.1: Voigt deconvolution of the ^{15}N CP/MAS NMR spectra of u-[^{13}C,^{15}N]-PCB-Cph1Δ2 in the Lumi-F (**A**) and Meta-F (**B**) recorded in a magnetic field of 17.6 T at 173 K and 203 K, respectively, using a spinning frequency of 8 kHz. The upper part compares the experimental NMR spectrum with the sum of the Voigt fits. The lower part gives the individual Voigt fits (dashed lines) and the residual spectra.

Chemical synthesis of ^{15}N21-PCB-Cph1Δ2

^{15}N21-PCB was synthesized by Dr. Bongards (Max-Planck-Institut für Bioanorganische Chemie, Mülheim an der Ruhr, Germany). The synthesis of 4*E*-4-ethylidene-3-methyl-5-thioxo(^{15}N)pyrrolidin-2-one (**6**) follows the synthesis scheme presented in Figure C.2.

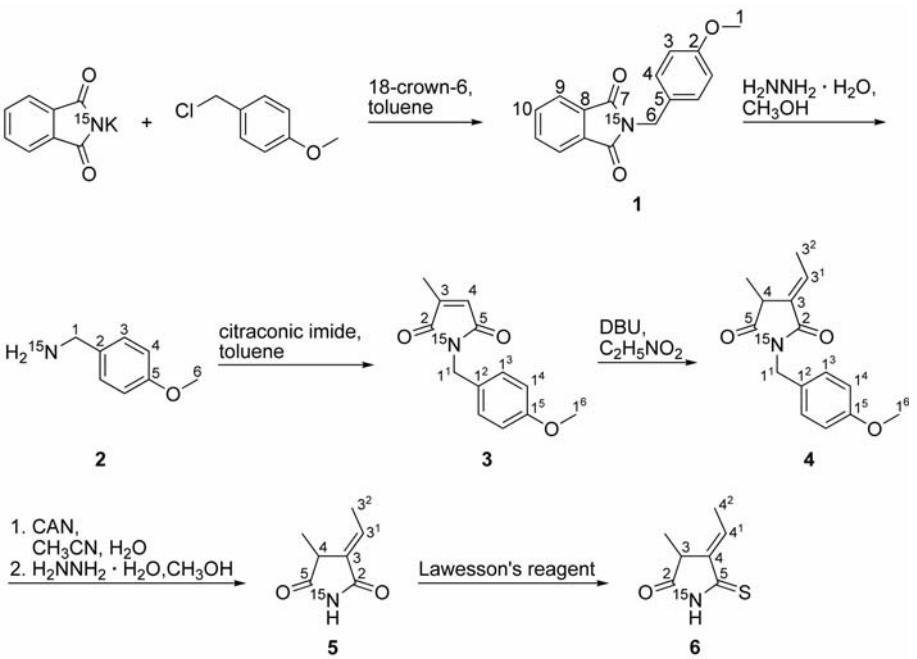

Figure C.2: Synthesis scheme applied for the the chemical synthesis of (4*E*)-4-ethylidene-3-methyl-5-thioxo(^{15}N)pyrrolidin-2-one (**6**).

2-(4-Methoxybenzyl)(^{15}N)-1*H*-isoindole-1,3(2*H*)-dione (**1**): A dispersion of ^{15}N-labeled potassium phthalimide (10.00 g, 53.7 mmol), 1-(chloromethyl)-4-methoxybenzene (6.1 mL, 44.8 mmol), and 18-crown-6 (1.18 g, 4.5 mmol) in toluene (58 mL) was stirred under argon at 100 °C for 5 h. After cooling to ambient temperature, water (150 mL) was added to the mixture. The resulting phases were separated, the aqueous phase was extracted with dichloromethane (4 × 50 mL) and the combined organic phases were dried over Na_2SO_4. Evaporation of the solvent and drying of the remaining pale yellow solid under reduced pressure yielded the reaction product **1** (12.01 g, 44.8

mmol, 100 %) which could be used in the following reaction without further purification.

1-(4-Methoxyphenyl)methan(^{15}N)amine (**2**): A dispersion of **1** (6.41 g, 23.9 mmol) and hydrazine hydrate (51 % hydrazine, 96 mL, 1.0 mol) in methanol (1.4 L) was refluxed for 5 h. After evaporation of the solvent under reduced pressure, the remaining oil was dissolved in dichloromethane (500 mL), washed with NaOH (1 M, 5 × 100 mL) and dried over Na_2SO_4. Evaporation of the solvent under reduced pressure yielded the reaction product **2** (3.20 g, 23.2 mmol, 97 %) as a yellow, clear oil.

In general, the preparation of **6** follows the synthetic route of the unlabeled ring ***A*** compound established by Kakiuchi *et al.* [139]. Transformation of the ^{15}N21-labeled ring ***A*** compound **6** to the full tetrapyrrole followed published procedures [140, 141].