



## Supporting Information

### **Erweiterung des Reaktionsspektrums: Nachweis des 5-Deazaflavin-Radikals durch photochemisch induzierte dynamische Kernpolarisation**

*J. Wörner, S. Panter, B. Illarionov, A. Bacher, M. Fischer, S. Weber\**

## Supporting Information

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# 1 Materials and Methods

## 1.1 Sample Preparation

L-Tryptophan and D<sub>2</sub>O (99.9 %) were purchased from Sigma-Aldrich. NaOD (40 % w/w, 99.5 % D) and DCl (22 % w/w, 99.5 % D) were purchased from Cambridge Isotope Laboratories. L-Tyrosine was purchased from Carl Roth GmbH + Co. KG. These chemicals were used without further purification. 5-DeazaFMN was synthesized following a procedure described by O'Brien *et al.* [1] and purified by high-performance liquid chromatography (HPLC) (LiChrospher RP-18 column, 150 mm × 20 mm) by using a linear 12–30 % gradient of methanol in water (flow rate: 10 mL/min, retention time: 18 min).

For the preparation of NMR samples, 5-deazaFMN was dissolved in D<sub>2</sub>O at a concentration of 0.8 mM. CIDNP samples were prepared to contain 0.20 mM of 5-deazaFMN and 5 mM of L-tryptophan or 1.3 mM of L-tyrosine. The samples were subsequently adjusted to the respective pH value by addition of small amounts of NaOD and DCl.

## 1.2 NMR and Photo-CIDNP Spectroscopy

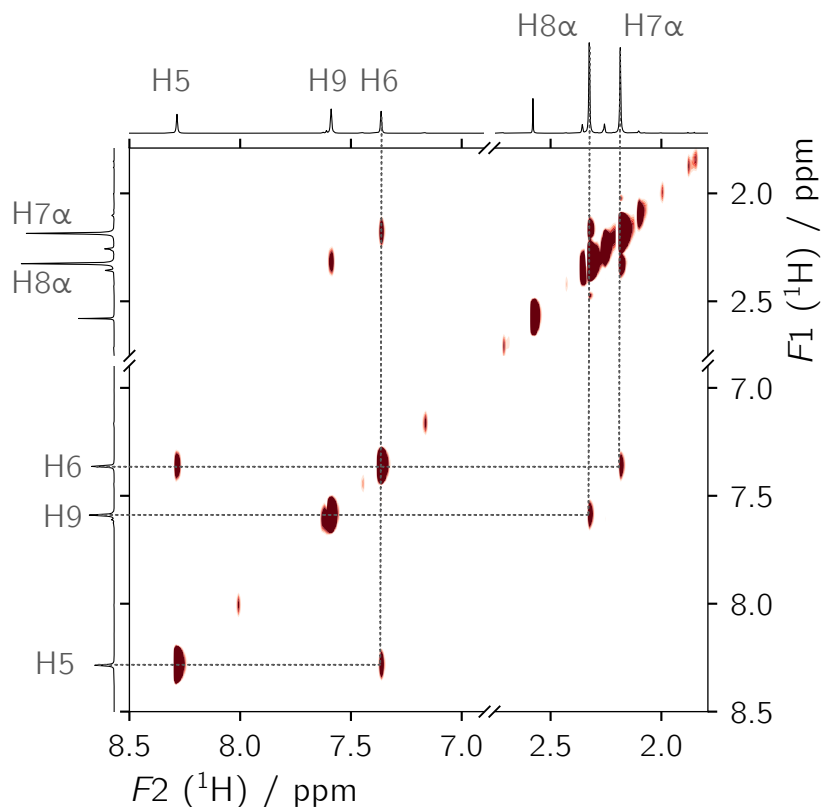
For the NMR experiments a Bruker Avance III HD 14.1 T NMR spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany) and a triple-resonance TXI probe optimized for proton experiments were used. All experiments were performed at 293 K. For the acquisition of thermally polarized <sup>1</sup>H spectra a standard pulse sequence was employed. Photo-CIDNP experiments were performed as previously described [2]. The samples were illuminated by insertion of an optical fiber (diameter: 1 mm, Thorlabs, Dachau, Germany) into the NMR tube via a coaxial insert (Wilmad WGS-5BL). For light excitation a system comprising a nanosecond-pulsed Nd:YAG laser (Surelite I, Continuum, Santa Clara, CA, USA) and a broadband OPO (Continuum OPO PLUS) was used. The excitation wavelength was set to 420 nm. For photo-CIDNP experiments a pulse sequence with a presaturation pulse train and destruction of thermal polarization prior to light excitation according to Goez *et al.* [3] as well as a destructive phase cycle was used. To obtain relative CIDNP intensities the CIDNP signals were fitted with the function achieving the highest correlation. A Lorentzian function was used for all signals with the exceptions of H9 at pH 6.6, H6 and H7α at pH 8.7 and H9 at pH 10.1 for which a Gaussian function was used.



## 1.3 Density Functional Theory Calculations

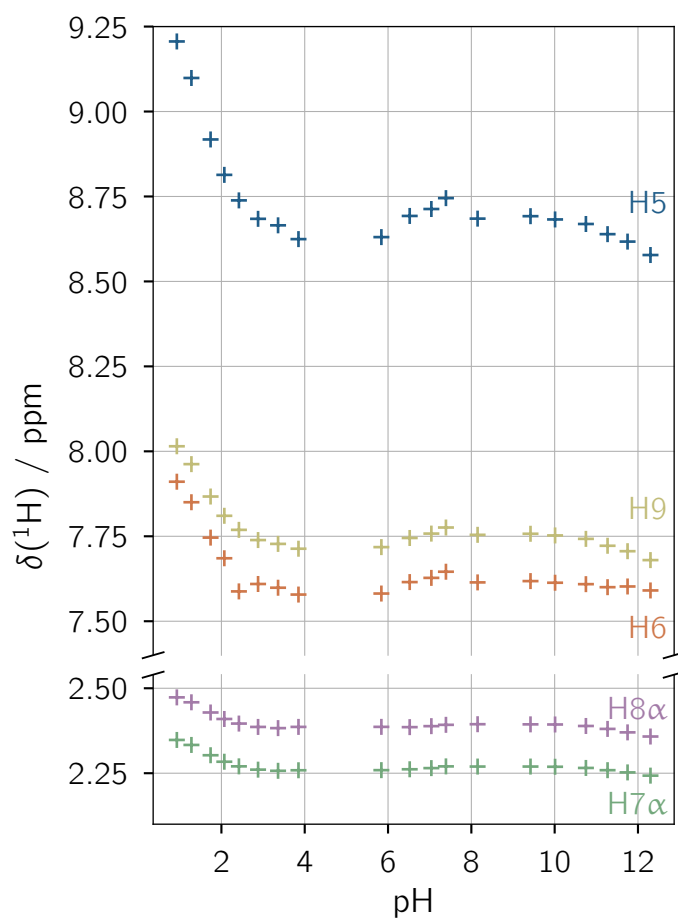
DFT calculations were performed with the ORCA program package (ORCA version 5.0) [4, 5]. Geometry optimizations were carried out by using the B3LYP functional [6] and the TZVP basis set [7]. Subsequent calculation of hyperfine coupling constants and  $\mathbf{g}$ -tensors were performed with the B3LYP functional and the IGLO-II basis set [8]. Additionally, the CPCM model [9] was used to simulate water solvation.

## 2 Assignments of $^1\text{H}$ Resonances in 5-DeazaFMN



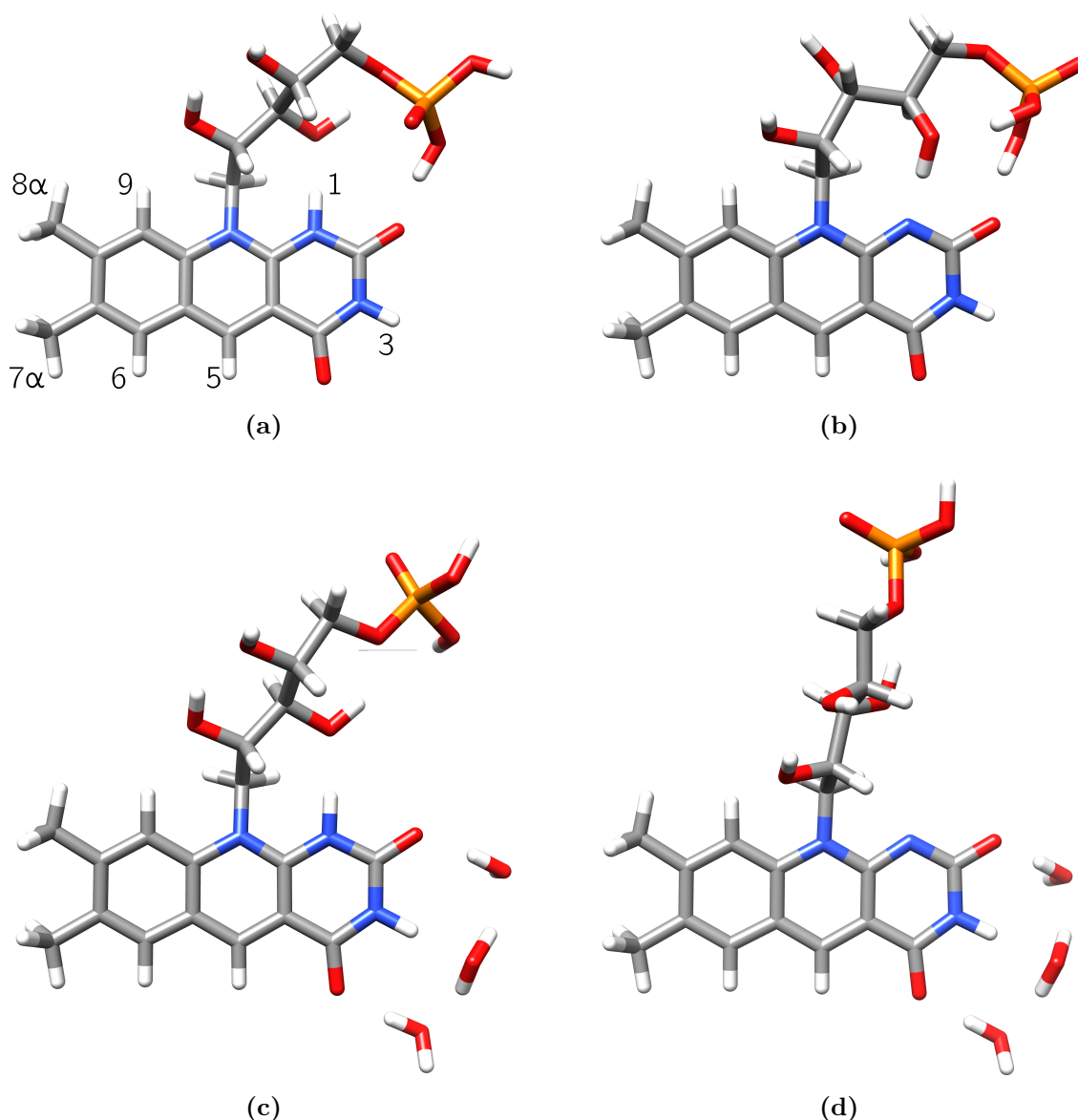
**Figure S1** Two-dimensional NOESY spectrum of 5-deazaFMN in  $\text{D}_2\text{O}$ . For acquisition a gradient-selected, phase-sensitive standard pulse sequence with solvent suppression by excitation sculpting was used [10–12] and a mixing time of 900 ms was chosen. Zero-filling was applied as well as line-broadening of 1.00 Hz and 0.30 Hz in  $F2$  and  $F1$  respectively prior to Fourier transformation. The spectrum was phase-corrected in  $F2$ . Cross-peaks between H7 $\alpha$  and H6, H6 and H5 as well as H8 $\alpha$  and H9 are marked.

### 3 pH Dependence of $^1\text{H}$ Resonances in 5-DeazaFMN



**Figure S2** pH dependence from pH 0.9 to 12.3 of  $^1\text{H}$  chemical shifts for 5-DeazaFMN. The values for nuclei of the aromatic 5-deazaFMN moiety are depicted. The chemical shifts were calibrated using the signal of residual HDO at 4.7 ppm.

## 4 Density Functional Theory Calculations of 5-DeazaFMN Radicals

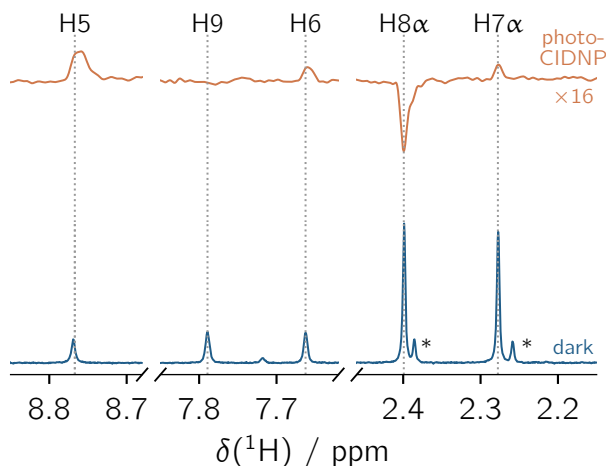


**Figure S3** Optimized structures of 5-deazaFMN radicals used for the calculation of isotropic hyperfine coupling constants and isotropic  $g$ -values: (a) 5-deazaFMN(H1)•, (b) 5-deazaFMN•-, (c) 5-deazaFMN(H1)• + 3 H<sub>2</sub>O, (d) 5-deazaFMN•- + 3 H<sub>2</sub>O. The numbering of <sup>1</sup>H of the aromatic 5-deazaFMN moiety according to IUPAC nomenclature is given in (a).

**Table S1** Calculated  $A_{\text{iso}}$  and isotropic  $g$  factors of anionic and neutral 5-deazaFMN radicals. Additionally, the two structures were modified by adding three  $\text{H}_2\text{O}$  molecules at specific positions. The structures are depicted in Fig. S3.  $A_{\text{iso}}$  are given in absolute and relative values. All relative values are normalized with respect to  $A_{\text{iso}}$  of H5.

	5-deazaFMN $\bullet^-$		5-deazaFMN(H1) $\bullet$		5-deazaFMN $\bullet^-$ + 3 $\text{H}_2\text{O}$		5-deazaFMN(H1) $\bullet$ + 3 $\text{H}_2\text{O}$	
$g_{\text{iso}}$	2.002799		2.002752		2.002822		2.002769	
	$A_{\text{iso,abs}}$ / MHz	$A_{\text{iso,rel}}$	$A_{\text{iso,abs}}$ / MHz	$A_{\text{iso,rel}}$	$A_{\text{iso,abs}}$ / MHz	$A_{\text{iso,rel}}$	$A_{\text{iso,abs}}$ / MHz	$A_{\text{iso,rel}}$
H5	-34.13	-1.00	-35.99	-1.00	-34.52	-1.00	-36.10	-1.00
H6	-11.55	-0.34	-11.23	-0.31	-11.38	-0.33	-11.30	-0.31
H7 $\alpha$	-4.18	-0.12	-4.06	-0.11	-4.14	-0.12	-4.23	-0.12
H8 $\alpha$	13.92	0.41	13.90	0.39	13.85	0.40	14.13	0.39
H9	2.92	0.09	3.30	0.09	3.14	0.09	3.54	0.10

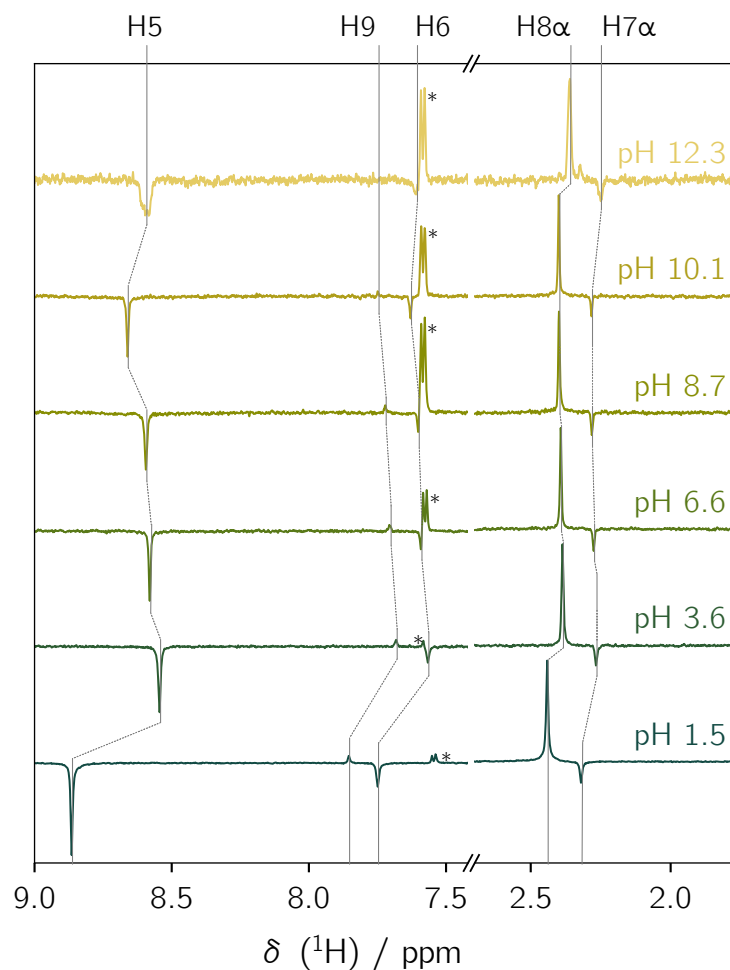
## 5 Photo-CIDNP Experiments



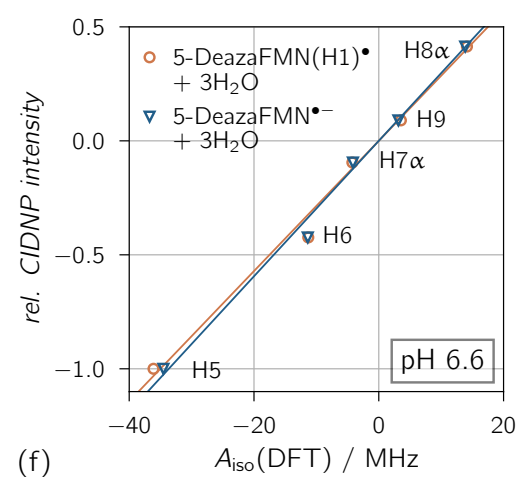
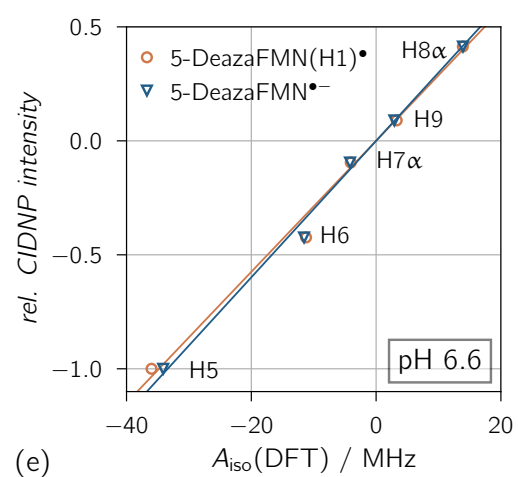
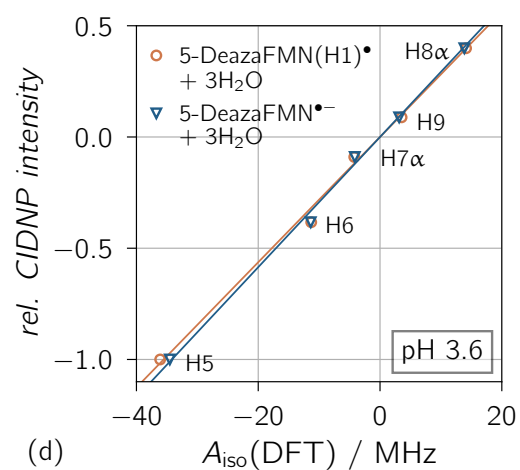
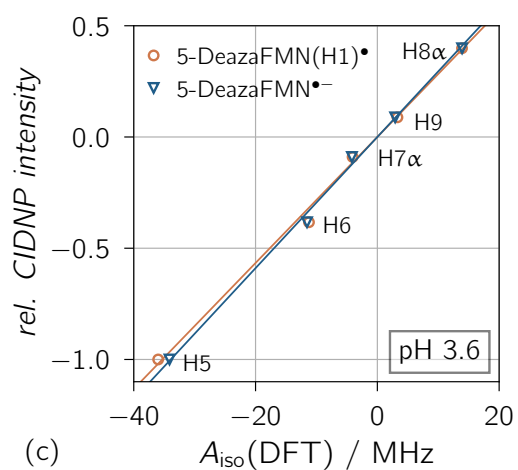
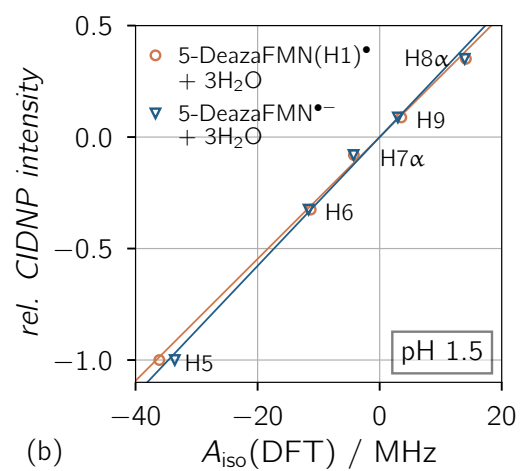
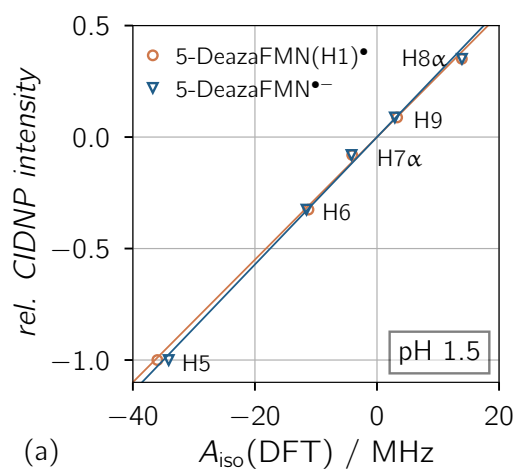
**Figure S4** Thermally polarized (blue) and photo-CIDNP (orange)  $^1\text{H}$  spectrum of 5-deazaFMN and L-tyrosine at pH 7.1. Only resonances of the aromatic moiety of 5-deazaFMN are shown. The sample was optically excited at 420 nm. The experiments were performed at 293 K with 128 and 48 scans, respectively. All resonances were referenced to the HDO signal at 4.7 ppm.

**Table S2** Relative  $A_{\text{iso}}$  of 5-deazaFMN radicals obtained from photo-CIDNP experiments (see Fig. S5). The values are given with the pH value of the investigated sample. All values are normalized with respect to  $A_{\text{iso}}$  of H5.

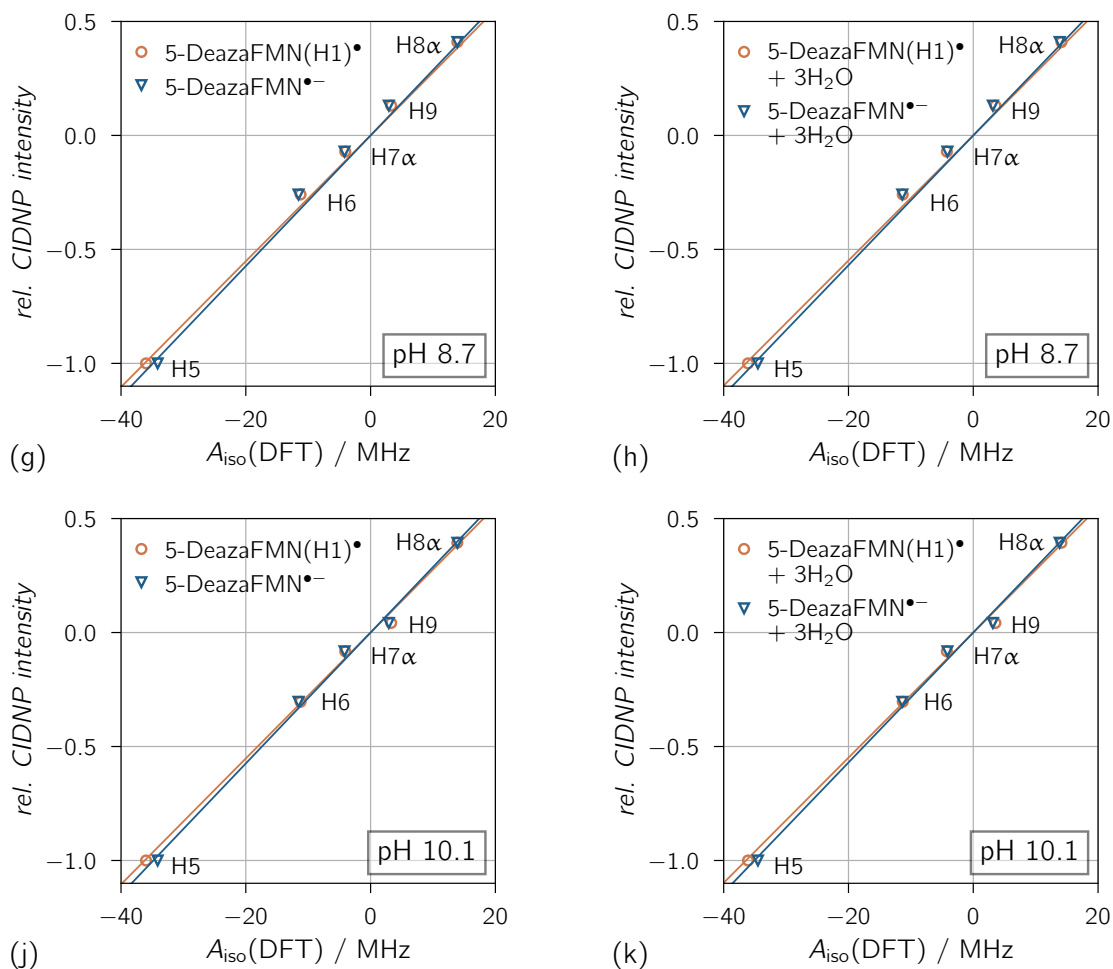
pH	1.5	3.6	6.6	8.7	10.1
H5	−1.00	−1.00	−1.00	−1.00	−1.00
H6	−0.33	−0.38	−0.42	−0.26	−0.30
H7 $\alpha$	−0.08	−0.09	−0.09	−0.07	−0.08
H8 $\alpha$	0.35	0.40	0.41	0.41	0.39
H9	0.09	0.09	0.09	0.13	0.04



**Figure S5** Photo-CIDNP experiments of 5-deazaFMN and L-tryptophan at pH values ranging from pH 1.5 to 12.3. The CIDNP signals are assigned to the respective proton of 5-deazaFMN. CIDNP signals of L-tryptophan are marked with asterisks. The samples were optically excited at 420 nm. The experiments were performed at 293 K with 1664, 512, 388, 1152, 512 and 384 Scans for pH 1.5 to pH 12.3 respectively.







**Figure S6** Linear regression fits of isotropic hyperfine coupling constants from DFT calculations and CIDNP experiments for the investigated 5-deazaFMN radical at (a) and (b): pH 1.5, (c) and (d): pH 3.6, (e) and (f): pH 6.6, (g) and (h): pH 8.7, (j) and (k): pH 10.1. CIDNP intensities were extracted from the experiments in Fig. S5. CIDNP intensities from the 5-deazaFMN radical at pH 12.3 were not evaluated due to poor signal-to-noise ratio. The fits were forced to go through the origin.

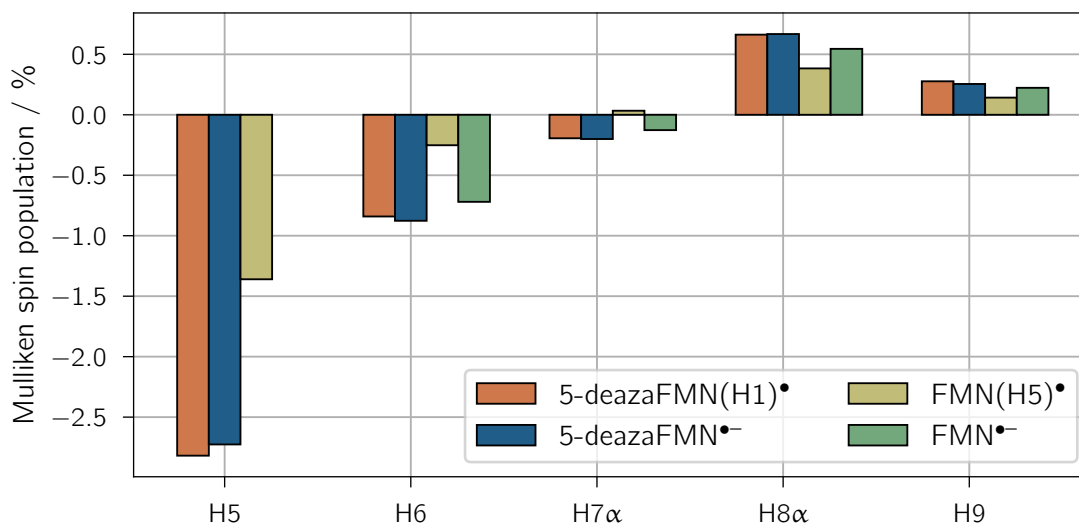
**Table S3** Results of linear regression plots of isotropic hyperfine coupling constants from DFT calculations and CIDNP experiments. Given are the slope and the coefficient of determination for 5-deazaFMN $\bullet^-$  and 5-deazaFMN(H1) $\bullet$ .

pH	5-deazaFMN $\bullet^-$		5-deazaFMN(H1) $\bullet$	
	slope / MHz $^{-1}$	$R^2$	slope / MHz $^{-1}$	$R^2$
1.5	0.0286	0.9960	0.0275	0.9980
3.6	0.0295	0.9973	0.0283	0.9954
6.6	0.0299	0.9937	0.0287	0.9899
8.7	0.0287	0.9959	0.0276	0.9989
10.1	0.0287	0.9960	0.0276	0.9968

**Table S4** Results of linear regression plots of isotropic hyperfine coupling constants from DFT calculations and CIDNP experiments. Given are the slope and the coefficient of determination for 5-deazaFMN $\bullet^-$  + 3 H<sub>2</sub>O and 5-deazaFMN(H1) $\bullet$  + 3 H<sub>2</sub>O.

pH	5-deazaFMN $\bullet^-$ + 3 H <sub>2</sub> O		5-deazaFMN(H1) $\bullet$ + 3 H <sub>2</sub> O	
	slope / MHz $^{-1}$	$R^2$	slope / MHz $^{-1}$	$R^2$
1.5	0.0284	0.9968	0.0273	0.9975
3.6	0.0293	0.9968	0.0281	0.9953
6.6	0.0297	0.9927	0.0286	0.9900
8.7	0.0285	0.9971	0.0274	0.9986
10.1	0.0285	0.9961	0.0274	0.9960

## 6 Mulliken Spin Populations of FMN and 5-DeazaFMN Radicals



**Figure S7** Comparison of  $^1\text{H}$  Mulliken spin populations of 5-deazaFMN(H1)• and 5-deazaFMN•<sup>-</sup> as well as FMN(H5)• and FMN•<sup>-</sup>.

**Table S5** Mulliken spin populations in % of  $^1\text{H}$  nuclei of FMN and 5-deazaFMN radicals in different protonation states. The values for  $^1\text{H}$  nuclei of the isoalloxazine moiety are given.

Proton	5-deazaFMN(H1)•	5-deazaFMN• <sup>-</sup>	FMN(H5)•	FMN• <sup>-</sup>
H5	-2.8187	-2.7259	-1.3602	0.0000
H6	-0.8409	-0.8764	-0.2518	-0.7198
H7 $\alpha$	-0.1943	-0.2004	0.0335	-0.1269
H8 $\alpha$	0.6626	0.6677	0.3836	0.5452
H9	0.2769	0.2548	0.1420	0.2230

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