Electronic Supplementary Material (ESI) for Chemical Communications.
This journal is © The Royal Society of Chemistry 2021

Supporting Information

Synthesis of Redox-Active Fluorinated 5-Hydroxytryptophans as Molecular Reporters for Biological Electron Transfer

Amanda Ohler,[b‡] Hanna Long,[a‡] Kei Ohgo,[a] Kristin Tyson,[a] David Murray,[a] Amanda Davis,[a] Chris Whittington,[a] Eli G. Hvastkovs,[a] Liam Duffy,[c] Alice Haddy,[c] Andrew L. Sargent,[a] William E. Allen,[a] and Adam R. Offenbacher[a*]

[b]Department of Chemistry, East Carolina University, Greenville, NC

[a]Department of Chemistry and Biochemistry, Ohio Northern University, Ada, OH

[c]Department of Chemistry and Biochemistry, University of North Carolina, Greensboro, NC

‡Contributed equally

*Correspondence: offenbachera17@ecu.edu

Methods

Materials. 5HOI, L-5HOW, and Fmoc-L-5HOW were obtained commercially from Sigma Aldrich. For this study, 4F-5HOI and 6F-5HOI were purchased as isolated products from eNovations (eNovation Chemicals LLC; Bridgewater, NJ). The purity of these chemicals (≥95%) were confirmed by ^{1}H NMR and HPLC. Synthesis of 4F-5HOI and 6F-5HOI can also be achieved as described previously,[1] using 2-fluoro-4-nitrophenol as a starting material.

UV-visible spectroscopy of F_n-5HOI and F_n-5HOW derivatives. For UV-vis studies of the small molecules, 200 μM solutions were prepared in 10 mM APB (acetate, phosphate, borate) buffer. For pKa determinations, KOH (elevated pH values)/HCl (reduced pH values) was used for titrations. UV/vis spectra were recorded for every 0.3-0.5 ΔpH (**Figure S3**). The changes in the absorbance of the red-shifted λ_{max} of the deprotonated species as a function of pH were fit to the equation: $y = \frac{10^{(x - pKa)}}{1 + 10^{(x - pKa)}}$ (where x is the pH value and y is the absorbance), using the normalized absorbance to account for slight changes in volume from the titrant.

At pH 7, absorbance spectra of serial dilutions were collected to determine molar extinction coefficients (ε) values: 5HOI (ε_{270nm} = 5690 $M^{-1}cm^{-1}$; ε_{290nm} = 3880 $M^{-1}cm^{-1}$); 4F-5HOI (ε_{260nm} = 6200 $M^{-1}cm^{-1}$; ε_{290nm} = 3120 $M^{-1}cm^{-1}$); 6F-5HOI (ε_{270nm} = 2180 $M^{-1}cm^{-1}$; ε_{290nm} = 2550 $M^{-1}cm^{-1}$). Note that L-5HOW has slightly red-shifted λ_{max} absorbances, ε_{275nm} = 5100 $M^{-1}cm^{-1}$ and ε_{295nm} = 4230 $M^{-1}cm^{-1}$, relative to 5HOI. The L-4F-5HOW and L-6F-5HOW derivatives exhibited nearly identical spectra and ε values as their indole derivatives. Using this approach, we determined a ε_{280} value of 5520 $M^{-1}cm^{-1}$ for natural tryptophan, which is comparable with the expected value of 5540 $M^{-1}cm^{-1}$.

Expression and Purification of TrpB synthases. The plasmids encoding the evolved TrpB synthase enzymes, Tm9D8*[2] and M145T N167D[3] The plasmids encoding these TmTrpB variants were a gift from Prof. Frances Arnold (CalTech). Each plasmid was transformed into *E. coli* BL21(DE3) cells. The enzymes were expressed in large scale (2 L) with 2xYT media supplemented with ampicillin. The cultures were grown at 37 °C to OD_{600} = 1.0, at which point the cultures were induced with 1 mM isopropyl-1-thio-β-galactopyranoside (IPTG). The temperature was adjusted to 18 °C and the cultures were incubated overnight. Cells were harvested by centrifugation and the pellet was stored at -80 °C until further use.

The thawed cell pellet was suspended 100 mL lysis buffer containing 10% glycerol, 50 mM $NaPO_4$, pH 7.0, and 50 mM NaCl, and 200 μL pyridoxal phosphate (PLP). Lysis was performed by sonication. The mixture was centrifuged at 18,000 x g to remove cell debris. The supernatant was incubated at 75°C for 10 minutes and the precipitated protein was removed by centrifugation. The protein was purified with an Ni-NTA column over a linear gradient from buffer A (20 mM Tris-HCl, 20 mM imidazole, 500 mM NaCl, pH 8.0) to buffer B (20 mM Tris-HCl, 200 mM imidazole, 500 mM NaCl, pH 8.0). The presence of the protein was checked with UV-Vis spectroscopy and SDS-PAGE. The purified proteins (**Figure S18**), confirmed by gel electrophoresis, were dialyzed overnight in 50 mM potassium phosphate, pH 8.0. The proteins were concentrated, flash-frozen in liquid N_2, and stored at -80 °C until needed. Typical yields were about 25 mg/L.

Optimization of enzymatic conversion of F_n-5HOI to F_n-L-5HOW. Small-scale (1 mL) reactions, initially performed for 5HOI, were prepared with solutions containing 20 mM of the 5-hydroxyindole derivative, 0.9 or 10 equivalents of L-serine, 40 μM or 100 μM PLP, and 5 μM tryptophan B synthase variant Tm9D8*, in 25 mM potassium phosphate

buffer, pH 7.0. The reaction times were varied from 4, 24, and 72 h at 37 °C and the reaction at 4 h was also tested at 55 °C. The small-scale reactions were monitored on a 4.6 x 50 mm C-18 silica column using acetonitrile with TFA, 0.1%/water: 0% acetonitrile for 1 min, 0% to 100% over 5 min, 100% for 1 min. 5HOI and its derivatives were seen to elute at *ca.* 11 minutes while 5HOW and its derivatives were seen to elute at *ca.* 5.7 minutes (**Figure S6C-F**). These retention times (RT) were validated using commercial 5HOI and L-5HOW, respectively (**Figure S6 A,B**). The integrations of the substrate and product peaks at 280nm were compared to determine the conversion. Once optimal conditions were determined for 5HOI, the reaction conditions were extended to 4F- and 6F-5HOI (**Figure S7D,E**). Note that the fluorinated derivatives of 5HOI were first dissolved in 50 μL DMSO (5% final concentration) before addition of aqueous buffer; DMSO did not greatly influence TrpB synthase reactivity.

Under the optimal conditions (4h, 37 °C), Tm9D8* was found to convert all F_n-5HOI derivatives to their corresponding UAAs in high yield (*cf.* **Figures S6B,C** and **S7C-E**).. For comparison, the application of another TmTrpB variant, M145T N167D, gave rise to high production yields for 5HOW and 6F-5HOW, with little-to-no conversion of 4F-5HOI to 4F-5HOW (*cf*. **Fig. S7F-J**). These data demonstrate a biocatalyst dependence.

Chemoenzymatic synthesis of Fmoc-F_n-5HOWs. Gram-scale syntheses of Fmoc-F_n-5HOW were prepared as follows: 0.27 g 5-hydroxyindole or 0.30 g 5-fluorinated hydroxyindole was dissolved in N_2-purged 100 mL of 20 mM potassium phosphate, pH 7.5, supplemented with 18 mM (0.9 eq.) L-serine, 5-10 μM Tm9D8* TrpB synthase. The reaction vessel was closed and incubated at 37 °C for 4 hours. TrpB synthase was removed by chelation with Ni-NTA beads. According to published protocols,[4] sodium bicarbonate (4 eq.) and Fmoc-succinimide (1 eq.) were

added to the reaction vessel with 100 mL 1,4-dioxane. The reaction was degassed and proceeded overnight with vigorous stirring at room temperature. The reaction vessel was then cooled to 0 °C and the solution was acidified (pH 2-3) with HCl and filtered. Extraction was completed with ethyl acetate and the products were washed with a brine solution. The solution was dried with sodium sulfate and filtered into a round bottom flask. The solvent was removed by low pressure. The product was purified by reverse phase HPLC (40:60:0.1 to 70:30:0.1 acetonitrile:water:TFA on semi-preparative C18 column). The HPLC solvent was removed by lyophilization. Note that the Fmoc-F_n-5HOW products exhibit RT of 13 minutes (**Figure S6H-J**), which is identical to the commercially available Fmoc-5HOW (**Figure S6G**). NMR spectra of newly synthesized compounds, Fmoc-4F-5HOW and Fmoc-6F-5HOW are presented in **Figure S8** and **S9**, respectively. HRMS data are presented in **Figure S10**.

Fmoc-5HOW: ^{1}H NMR (400 MHz, DMSO-d_6), δ = 12.65 (s, 1H), 10.53 (s, 1H), 8.57 (s, 1H), 7.87 (d, 2H), 7.65 (m, 2H), 7.0-7.4 (m, 7H), 6.86 (d, 1H), 6.61 (d, 1H), 4.19 (m, 3H), 2.96 (m, 2H). MS, observed: 442.19; predicted ($C_{26}H_{22}N_2O_5$): 442.15. These data match commercial compounds.

Fmoc-4F-5HOW: ^{1}H NMR (400 MHz, DMSO-d_6), δ = 12.56 (s, 1H), 10.77 (s, 1H), 8.78 (s, 1H), 7.87 (d, 2H), 7.65 (m, 2H), 7.2-7.4 (m, 3H), 7.06 (s, 1H), 6.96 (d, 1H), 6.72 (s, 1H), 4.18 (m, 3H), 3.0-3.3 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6), δ = 174.33, 156.45, 145.69, 144.24, 143.33, 141.13, 136.40, 133.06, 128.08, 127.53, 125.7, 125.36, 120.55, 113.84, 108.33, 107. 48, 66.06, 55.59, 47.05, 28.51. ^{19}F NMR (376 MHz, DMSO-d_6), δ = -149.85 ppm. HRMS, observed: 461.1515 $(M+H)^+$; predicted ($C_{26}H_{21}FN_2O_5$): 461.1507. ^{1}H and ^{13}C NMR spectra of Fmoc-4F-5HOW are also presented in **Figure S8**.

Fmoc-6F-5HOW: ^{1}HNMR (400 MHz, DMSO-d_6), δ = 12.66 (s, 1H), 10.60 (s, 1H), 9.00 (s, 1H), 7.87 (d, 2H), 7.66 (m, 2H), 7.2-7.4 (m, 4H), 7.08 (s, 3H), 4.20 (m, 4H), 2.9-3.1 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6), δ = 174.23, 156.47, 150.89, 148.56, 144.23, 141.13, 139.31, 128.08, 127.53, 125.7, 125.63, 124.47, 123.57, 120.55, 109. 85, 104.91, 66.10, 55.14, 47.05, 27.40. ^{19}F NMR (376 MHz, DMSO-d_6), δ = -141.38 ppm. HRMS, observed: 461.1515 $(M+H)^+$; predicted ($C_{26}H_{21}FN_2O_5$): 461.1507. ^{1}H and ^{13}C NMR spectra of Fmoc-6F-5HOW are also presented in **Figure S9**.

Peptide synthesis. The 12-mer peptide, H_2N-Arg-F_n5HOW-Val-Glu-Val-Asn-Gly-Orn-Lys-Ile-Phe-Gln-$CONH_2$, was synthesized on Rink Amide AM resin using standard Fmoc chemistry, using an automated peptide synthesizer. The peptide was cleaved from the resin using a mixture of TFA/1,3-dimethoxybenzene/TES (95:2.5:2.5) and was precipitated in cold diethyl ether. The peptide was purified by reverse phase HPLC on a semipreparative C18 column using a linear gradient from 0.1% TFA to 99.9% acetonitrile/0.1% TFA. LC-MS observed: 1504.11, predicted for $C_{69}H_{109}N_{21}O_{17}$: 1503.83 (L-5-HOW containing peptide). The presence of F_n-5HOW derivatives were further confirmed by UV-visible spectroscopy (**Figure S12**).

Incorporation of L-5HOW in Azurin. The DNA encoding the tyrosine-deficient variant of *Pseudomonas aeruginosa* azurin (W48/Y72F/Y108F) was mutated with so that W48 was substituted for the AMBER stop codon, TAG, by GenScript (Piscataway, NJ). The pET-9a plasmid encoding this gene was co-transformed into *E. coli* BL21 (DE3) pLysS with an evolved tryptophanyl tRNA synthase/tRNA pair (acquired from Prof. Peter Schultz) and expressed in 2xYT media supplemented with 1 mM L-5HOW. Expression was induced with 1 mM IPTG once the optical density (OD_{600}) reached 0.9-1.2. The temperature was reduced to 18°C and incubated overnight.

Incorporation of 4F- and 6F-L-5HOW in Azurin. The fluorinated 5-hydroxytryptophan derivatives were incorporated into azurin using a Trp auxotroph of *E. coli* W3110TrpA33(DE3)/pLysS,[5] provided by Prof. Bridgette Barry (Georgia Tech), and the plasmid encoding the wild-type gene of azurin. A starter culture was grown in 50 mL M9 minimal media, which was supplemented with 80 mg L^{-1} L-tryptophan, overnight at 37 °C. Fresh 900 mL M9 media containing 80 mg L^{-1} L-Trp was inoculated with 20 mL of overnight culture and incubated at 37°C until an OD_{600} of 0.9–1.1 was reached. The cells were collected by low-speed centrifugation (3,000 × g, 10 min, room temperature) and gently resuspended in fresh 900 mL M9 media, supplemented with 50-80 mg L^{-1} 4F- or 6F-L-5HOW (see below). The culture was incubated at 37 °C for 45 min, and azurin expression was induced with 1 mM IPTG.

Azurin protein was purified from cell pellet, as previously described.[6, 7] Briefly, the periplasmic fraction of *E. coli* cell pellet was purified by incubation (37°C, 1 h) in resuspension buffer (20 mM potassium phosphate, 2 mM $MgSO_4$, pH 7) supplemented with lysozyme/DNAse I. Cell debris was removed by centrifugation and the solution was adjusted to pH 4.5 using sodium acetate buffer. After removal of aggregate, copper sulfate pentahydrate was added to 10 mM. The protein solution was dialyzed overnight at 4 °C in 1 mM sodium acetate, pH 4.5. Azurin was purified to homogeneity, as assessed by a ~14 kDa band in SDS-PAGE (**Figure S13**) using a HiTrap SP column attached to AKTA FPLC.

Circular Dichroism: Protein and Peptide Folding and Stability. The folding of the beta hairpin peptide was demonstrated using CD measurements (Jasco CD spectrometer J-815). The CD spectra of the synthetic peptides were analyzed at pH 7 in 10 mM phosphate buffer and at pH 11 in borate buffer. The peptide concentration was 200 μM. Note: the HT did rise above 600 V only in the case of elevated pH and temperature. For each condition, the CD spectra of the peptides

were recorded at 25 °C, before and after a 90 °C 'melt' (**Figure S11**). Briefly, upon completion of the first 25 °C CD spectrum, the temperature was raised to 90 °C and held for 5 minutes. The 90 °C 'melt' CD spectrum was collected and then allowed to cool.

The CD spectrum was collected for azurin protein (25 μM) samples at 25 °C in 10 mM phosphate, pH 7 buffer (**Figure S14**). For stability measurements for azurin, the wavelength was set to 220 nm and the temperature ranged from 25-90 °C. Measurements were taken at an interval of 2 °C with a ramp rate of 0.3 °C/min and D.I.T. of 8 sec.

Electrochemistry. Solutions of N-acetylated tryptophan, N-acetylated tyrosine, F_n-5HOI, and 5HOW-containing peptides were prepared at 0.2 mM concentrations in 10 mM APB (acetate, phosphate, borate) buffer, supplemented with 200 mM KCl. The solutions were titrated to a range of pH values, from 2 to 13 (by 0.3-0.5 pH increments) using 1 M KOH or 1 M HCl. Note: the peptide solutions were only analyzed at pH 7. A CH Instruments (Austin, TX) 600A potentiostat, pyrolytic graphite (PG) working, Pt counter, and saturated calomel (saturated KCl) reference electrodes were used for analysis of these solutions. Square wave voltammetry (SWV) scans were performed with the following parameters: potential scan range 0 V to 1.1 V, increments of 0.004 V, amplitude of 0.025 V, frequency of 15 Hz, quiet time of 2 sec, and a sensitivity of 1×10^{-5} A.

For analysis of the electrochemical potentials of 5HOW in azurin, the protein was immobilized to PG electrodes were prepared using the layer-by-layer (LbL) method, as previously described.[7] The data were collected with a CH Instruments 600A potentiostat with Pt counter and saturated calomel (saturated KCl) reference electrode. SWV scans were performed with the following parameters: potential scan range 0.0 to 1.0 V, step increments of 0.004 V, amplitude of 0.025 V, frequency of 30 Hz, quiet time of 2 sec, and a sensitivity of 1×10^{-5} A. Electrochemical

data analysis was performed using Origin Pro software. SWV data were corrected for NHE and background subtracted.

Electron Paramagnetic Resonance (EPR) Spectroscopy. EPR spectra on samples in clear fused quartz sample tubes were recorded at 77 K on a Bruker Instruments (Billerica, MA) EMX 10/12 X-band EPR spectrometer, equipped with a ER4116DM cavity and a Wilmad (Buena, NJ) liquid nitrogen dewar. Solutions of Y, W, and F_n-5HOI were prepared at 50 mM concentrations in 10 mM borate buffer, pH 11.0. The peptide concentrations were 4 mM in the same buffer. Azurin samples (final concentration, 0.3-1 mM) were mixed in an anaerobic chamber with 5 mM $[Co^{III}(NH_3)_5Cl]Cl_2$ (exogenous electron acceptor) in 20 mM potassium phosphate, pH 7.5, according to references.[8, 9]

Tyrosyl or tryptophanyl radicals were generated by photolysis with the 266 nm output from a Continuum Surelite II-10 Nd:YAG laser. The pulse energy at the site of excitation was 8-11 mJ/pulse at a frequency of 10 Hz, and approximately 50 (amino acids and peptides) and 1,200 (azurin) flashes were employed. The EPR spectrometer conditions were as follows: microwave frequency, 9.63 GHz; microwave power, 200 μW; modulation frequency, 100 kHz; modulation amplitude, 2 G; time constant, 81.92 ms; conversion time, 81.92 ms; resolution, 1024. For W48•, the microwave power was lowered to 20 μW to avoid saturation of the EPR signal.

Density Functional Theory (DFT) Calculations. Optimized molecular geometries were evaluated within the Gaussian '09 Program Suite[10] using a B3LYP[11, 12] density functional and a 6-31+G* basis set. Frequency calculations were performed to not only characterize the optimized stationary points but also to utilize the zero-point energy when calculating ionization potentials.[13] Electrostatic potential maps were generated with the Gauss View 5.0.9 program,[14] and spin

densities were plotted with the Spartan 18 program package.[15] All structures and surfaces were evaluated with larger basis sets, including the 6-311+G(2df,2p) set and the aug-cc-pvtz set, but no significant differences were noted. In all cases, no solvent approximation was employed in order to be consistent with previous reports.[13, 16] The impact of the polarized continuum model of solvation[17, 18] was investigated regarding the calculated ionization potentials in a water dielectric environment, and the results are listed in the **Table S2.** Cartesian coordinates for the geometry optimized F_n-5HOI/5HOW structures are presented in **Table S6**.

References

1. M. Makosza, W. Danikiewicz and K. Wojciechowski, *Liebigs Ann. Chem.*, 1988, **3**, 203-208.
2. C. E. Boville, D. K. Romney, P. J. Almhjell, M. Sieben and F. H. Arnold, *J. Org. Chem.*, 2018, **83**, 7447-7452.
3. J. Murciano-Calles, D. K. Romney, S. Brinkmann-Chen, A. R. Buller and F. H. Arnold, *Angew. Chem.* , 2016, **55**, 11577-11581.
4. Z.-J. Yao, Y. Gao, J. H. Voigt, H. Ford Jr. and T. R. Burke Jr., *Tetrahedron*, 1999, **55**, 2865-2874.
5. A. R. Offenbacher, C. V. Pagba, B. C. Polander, U. Brahmachari and B. A. Barry, *ACS Chem. Biol.*, 2014, **9**, 891-896.
6. M. Piccioli, C. Luchinat, T. J. Mizoguchi, B. E. Ramirez, H. B. Gray and J. H. Richards, *Inorg. Chem.*, 1995, **34**, 737-742.
7. K. J. Tyson, A. N. Davis, J. L. Norris, L. J. Bartolotti, E. G. Hvastkovs and A. R. Offenbacher, *J. Phys. Chem. Lett.*, 2020, **11**, 2408-2413.
8. J. E. Miller, C. Gradinaru, B. R. Crane, A. J. Di Bilio, W. A. Wehbi, S. Un, J. R. Winkler and H. B. Gray, *J. Am. Chem. Soc.*, 2003, **125**, 14220-14221.
9. H. S. Shafaat, B. S. Leigh, M. J. Tauber and J. E. Kim, *J. Am. Chem. Soc.*, 2010, **132**, 9030-9039.
10. M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, G. A. Petersson, H. Nakatsuji, X. Li, M. Caricato, A. V. Marenich, J. Bloino, B. G. Janesko, R. Gomperts, B. Mennucci, H. P. Hratchian, J. V. Ortiz, A. F. Izmaylov, J. L. Sonnenberg, Williams, F. Ding, F. Lipparini, F. Egidi, J. Goings, B. Peng, A. Petrone, T. Henderson, D. Ranasinghe, V. G. Zakrzewski, J. Gao, N. Rega, G. Zheng, W. Liang, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, K. Throssell, J. A. Montgomery Jr., J. E. Peralta, F. Ogliaro, M. J. Bearpark, J. J. Heyd, E. N. Brothers, K. N. Kudin, V. N. Staroverov, T. A. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. P. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, J. M. Millam, M. Klene, C.

Adamo, R. Cammi, J. W. Ochterski, R. L. Martin, K. Morokuma, O. Farkas, J. B. Foresman and D. J. Fox, *Journal*, 2016.
11. C. Lee, W. Yang and R. G. Parr, *Phys. Rev. B*, 1988, **37**, 785-789.
12. A. D. Becke, *J. Chem. Phys.*, 1993, **98**, 5648-5652.
13. T. Liu, P. R. Callis, B. H. Hesp, M. de Groot, W. J. Buma and J. Broos, *J. Am. Chem. Soc.*, 2005, **127**, 4104-4113.
14. M. J. Frisch, H. P. Hratchian, R. D. Dennington II, T. A. Keith and J. Millam, *Journal*, 2009.
15. W. Herhe and S. Ohlinger, *Journal*, 2019.
16. R. Sibert, M. Joscowicz, F. Porcelli, G. Veglia, K. Range and B. A. Barry, *J. Am. Chem. Soc.*, 2007, **129**, 4393-4400.
17. S. Miertus, E. Scrocco and J. Tomasi, *Chem. Phys.*, 1981, **55**, 117-129.
18. S. Miertus and J. Tomasi, *Chem. Phys.*, 1982, **65**, 239-245.
19. W. A. Remers and R. K. Brown, *Indoles, Part 1: Chemistry of Heterocyclic Compounds 25*, Wiley, New York, 1974.

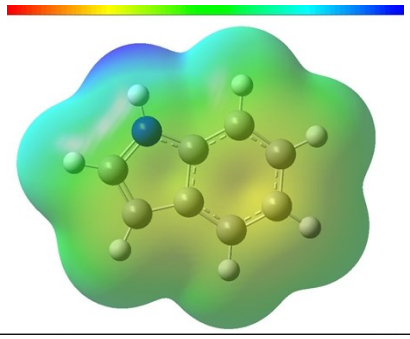
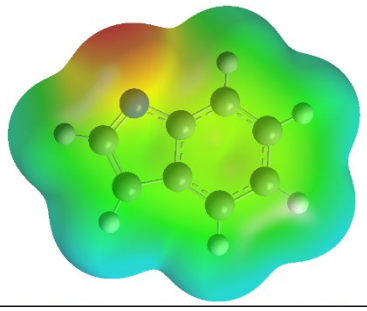
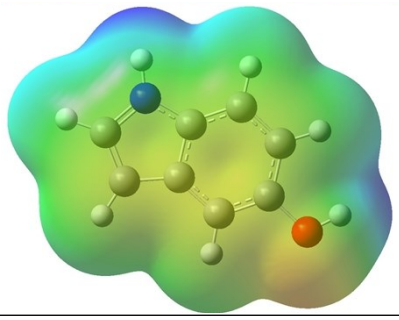
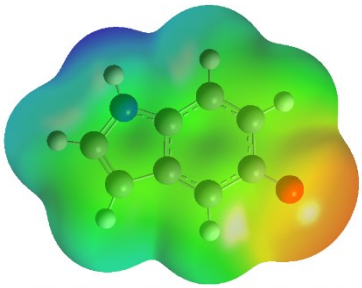
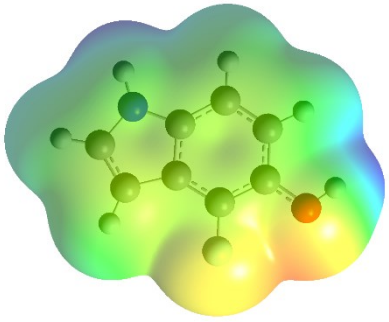
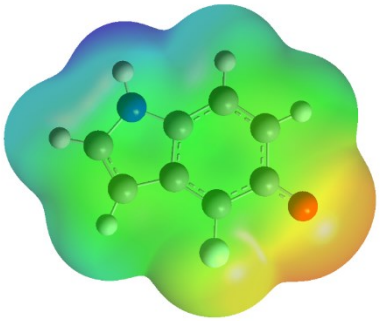
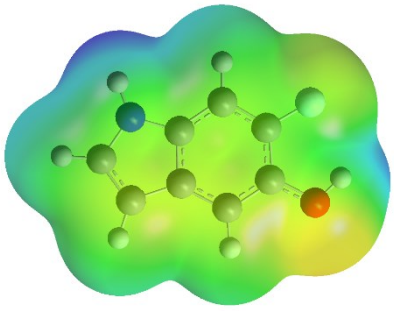
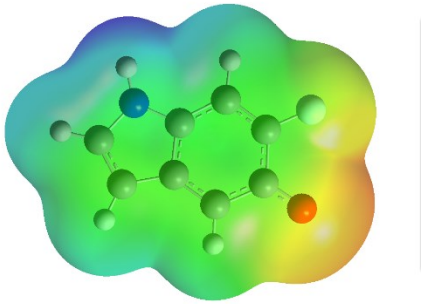
	Singlet (ground state)	Radical
Indole		
5HOI		
4F-HOI		
6F-HOI		

Figure S1. Electrostatic maps of the ground states (singlet) and radical states of indole and F_n-5HOI. Greater negative charge is represented by red, greater positive charge by blue. These EP maps are not dependent upon basis set used (*cf.* **Figure S16**).

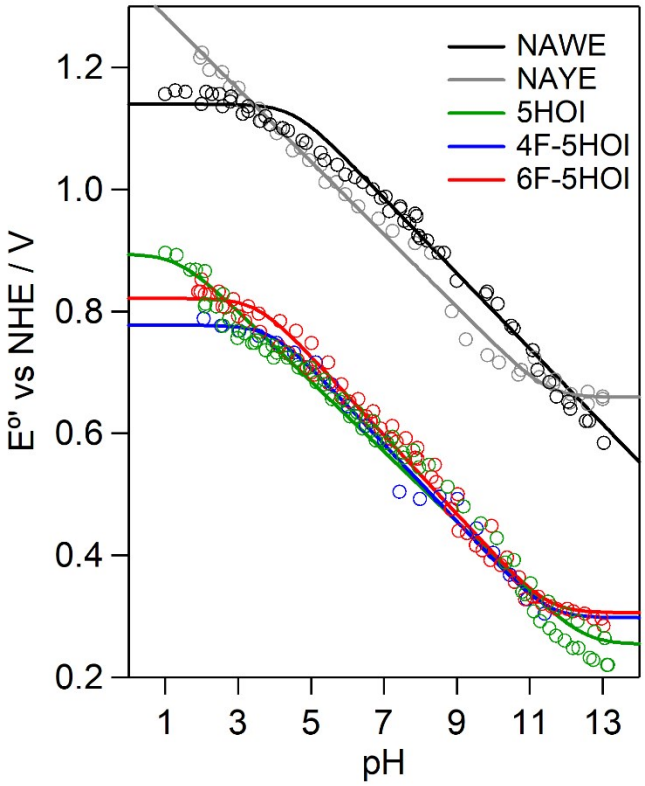


Figure S2. Pourbaix plots of N-acetylated tyrosine (NAYE), N-acetylated tryptophan (NAWE), and F_n-5HOIs. The SWV parameters are listed in the Methods section. The slopes of pH-dependent regions are 59-61 mV/pH. Potentials at pH 7 are listed in **Tables S2** and **S5**.

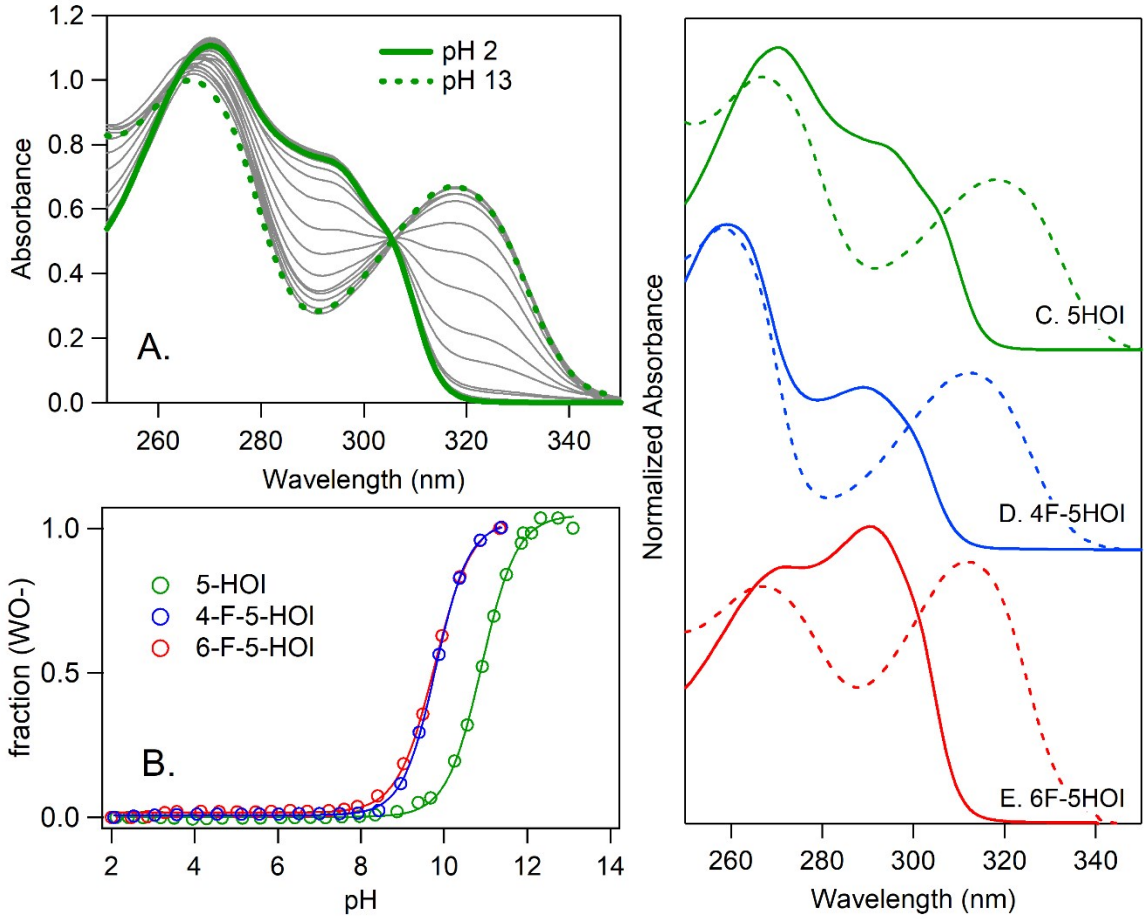


Figure S3. UV-visible spectra for 5HOI as a function of pH (A). The spectrum of the protonated form is in bold solid green line and the deprotonated form (proton lost from the hydroxyl functional group) is represented by the bold dashed line. The changes in the absorbance profile as a function of pH are shown in gray. In (B), the fit, using the equation: $y = \frac{10^{(x - pKa)}}{1 + 10^{(x - pKa)}}$, to the normalized absorbance at 320 nm for 5HOI in (A) is represented as the green trace. The data from pH titrations to 4F-5HOI and 6F-HOI are represented by blue and red colors, respectively. The pK_a values are presented in **Table S2**. Panels (C-E) provide the spectra for the protonated (solid lines) and deprotonated (dashed lines) species for 5HOI, 4F-5HOI and 6F-HOI.

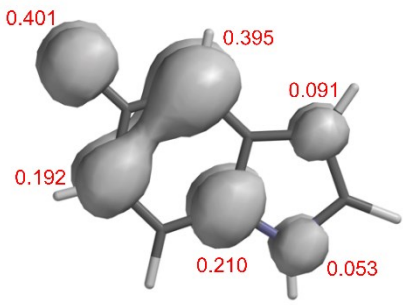
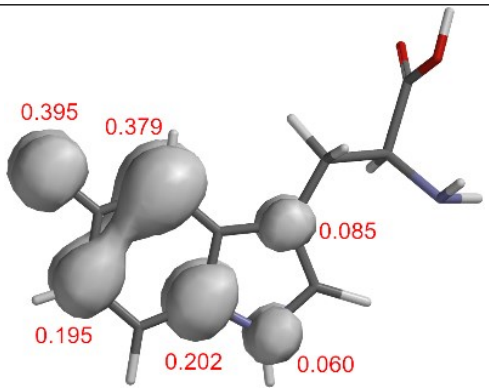
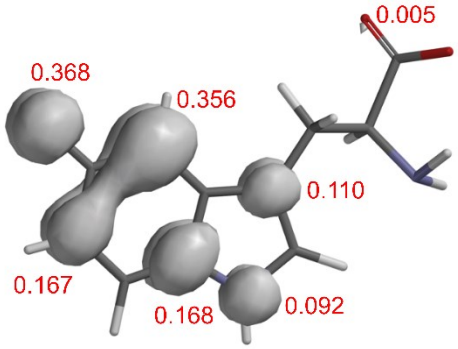
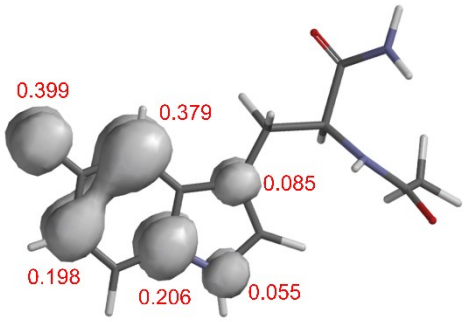
5HOI•	
5HOW• (protonated carboxylate)	
5HOW• (deprotonated carboxylate)	
Ac-5HOW•-NH_2	

Figure S4. Dependence of the Mulliken spin densities upon the presence and nature of the backbone for 5HOI•/5HOW•.

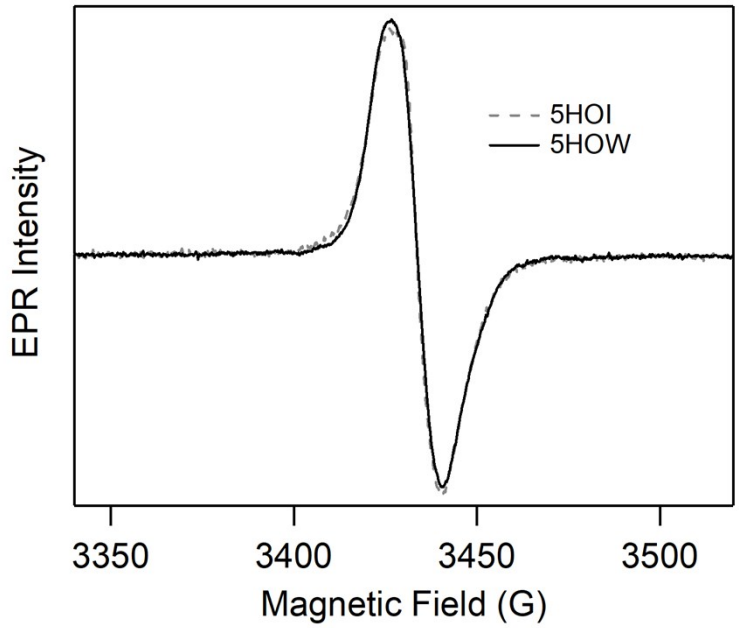


Figure S5. EPR spectra of 5HOI (dashed gray line) and 5HOW (solid black line) radicals were collected at 77 K, post UV photolysis from a pulsed Nd-YAG laser. The solutions were prepared at 50 mM in 50 mM sodium borate, pH 11.

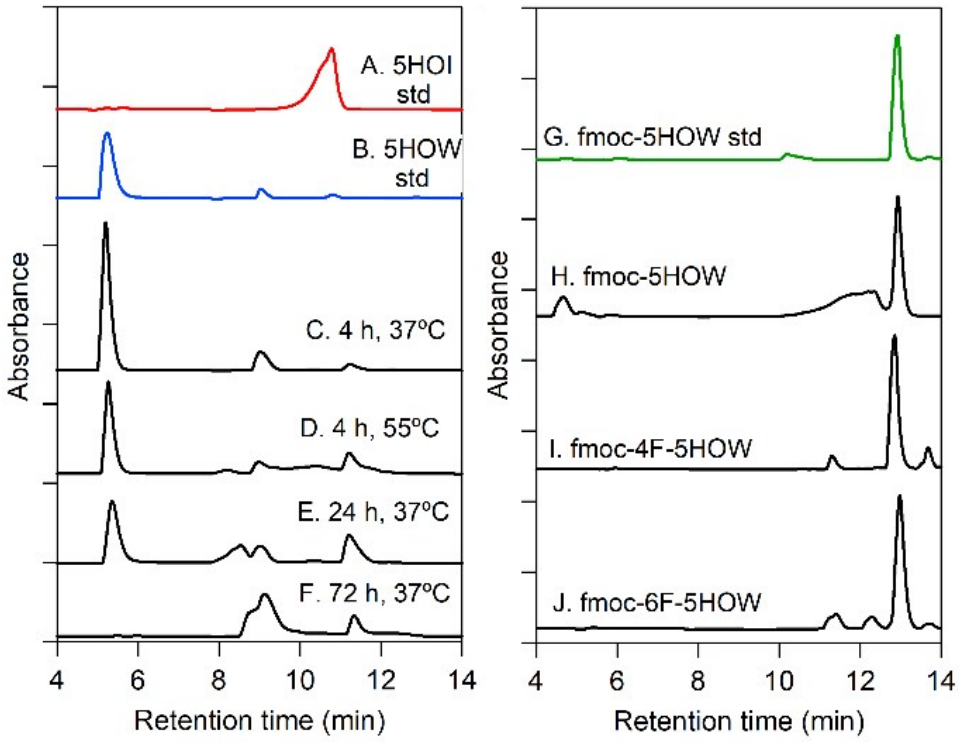


Figure S6. HPLC traces for the as-isolated products from F_n-5HOI to L-F_n-5HOW reactions (C-F) and as-isolated products of Fmoc-protected Fn-5HOW (H-J). See SI for more details. A,B, and G are commercial standards.

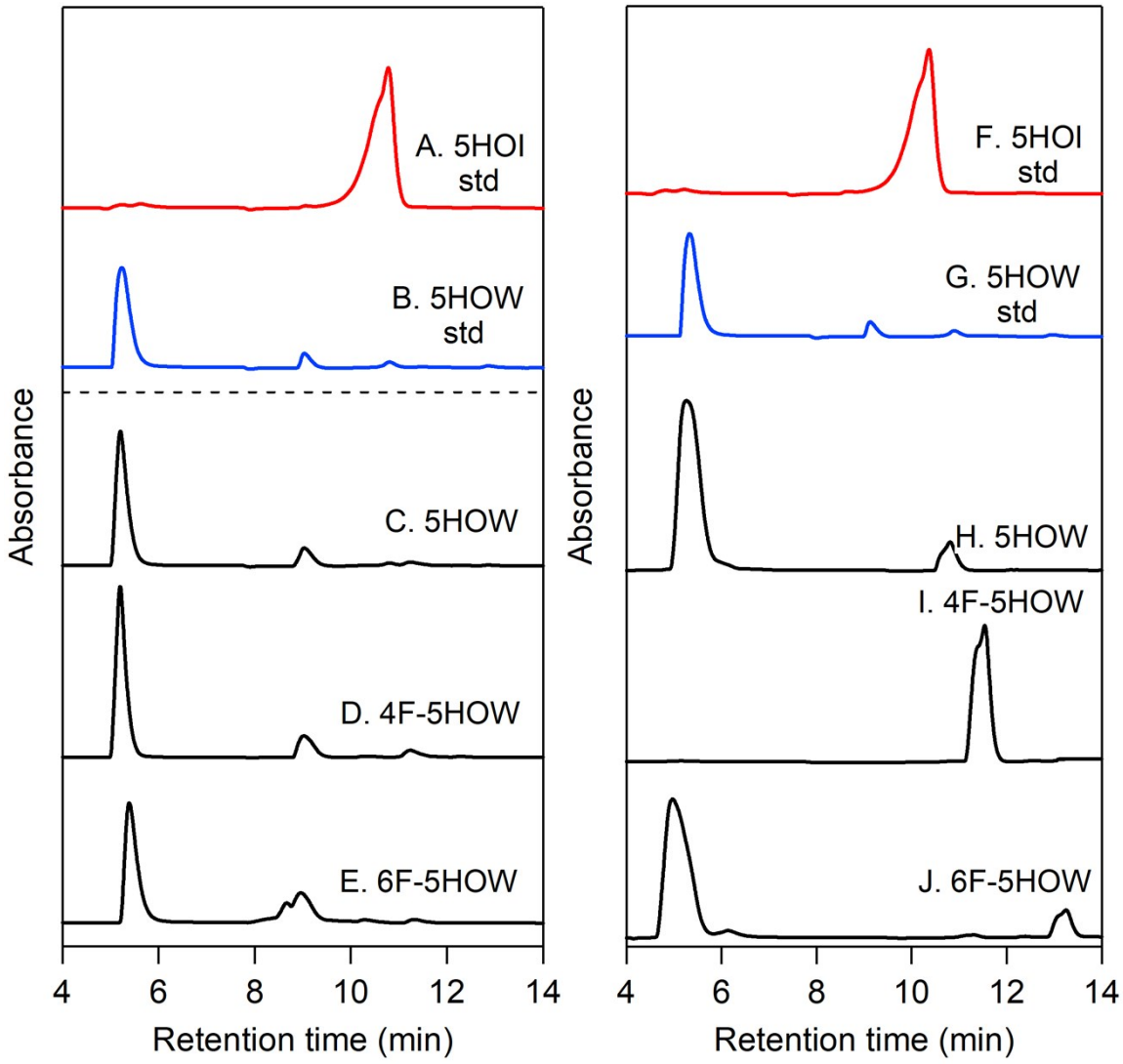


Figure S7. HPLC traces for small-scale reactions of F_n-5HOI to F_n-5HOW using (*left*) Tm9D8* or (*right*) M145T N167D TmTrpB synthases. The red and blue traces represent commercial standards for 5HOI and 5HOW, respectively. The solution conditions were: 20 mM hydroxyindole derivative, 0.9 equivalents L-Ser, 40 μM PLP, 5 μM TrpB enzyme in 25 mM phosphate, pH 7.5. The reactions were carried out at 37°C for 4 hours.

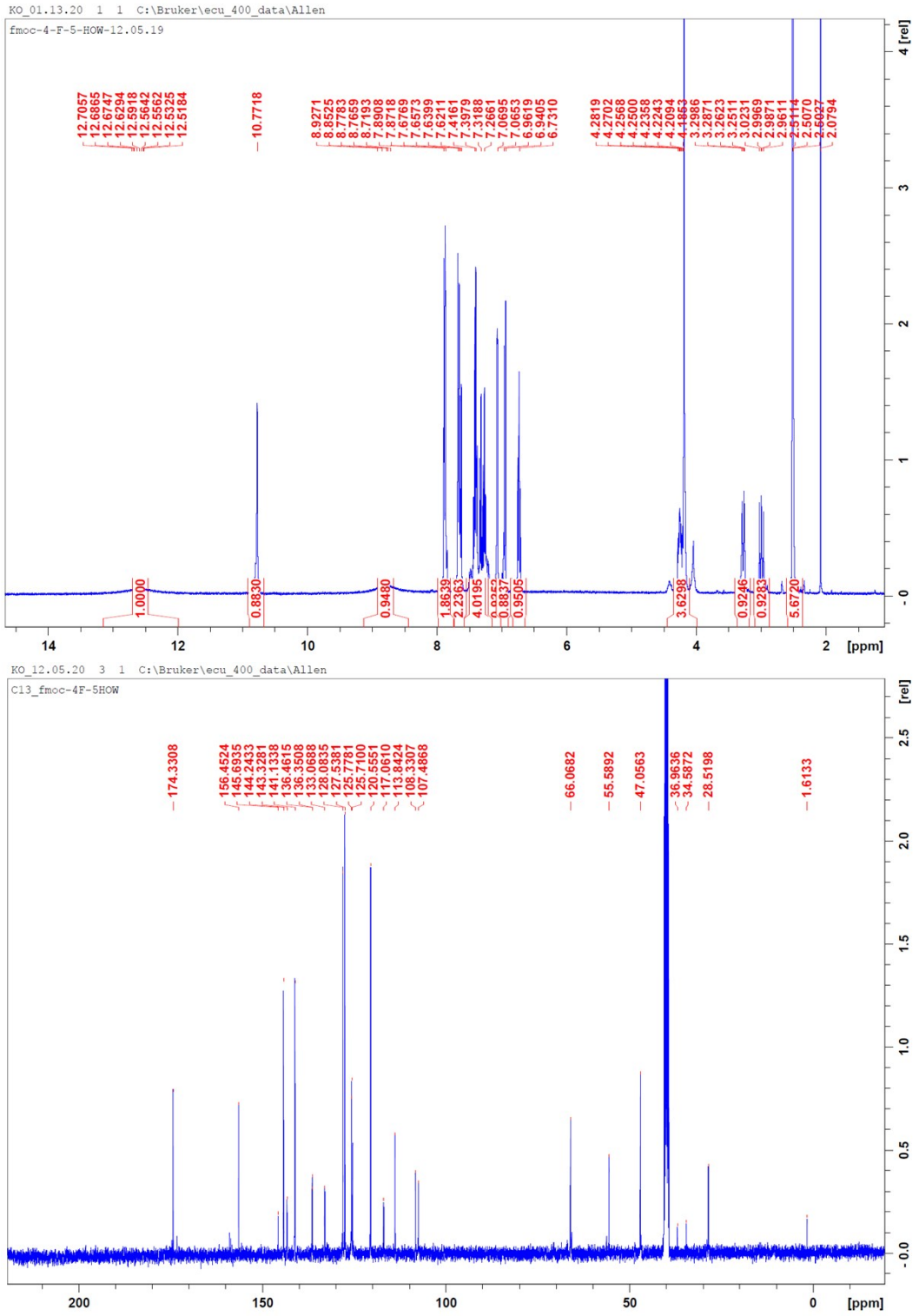


Figure S8. ^{1}H (top) and ^{13}C (bottom) NMR spectra of Fmoc-4F-5HOW in DMSO-d_6.

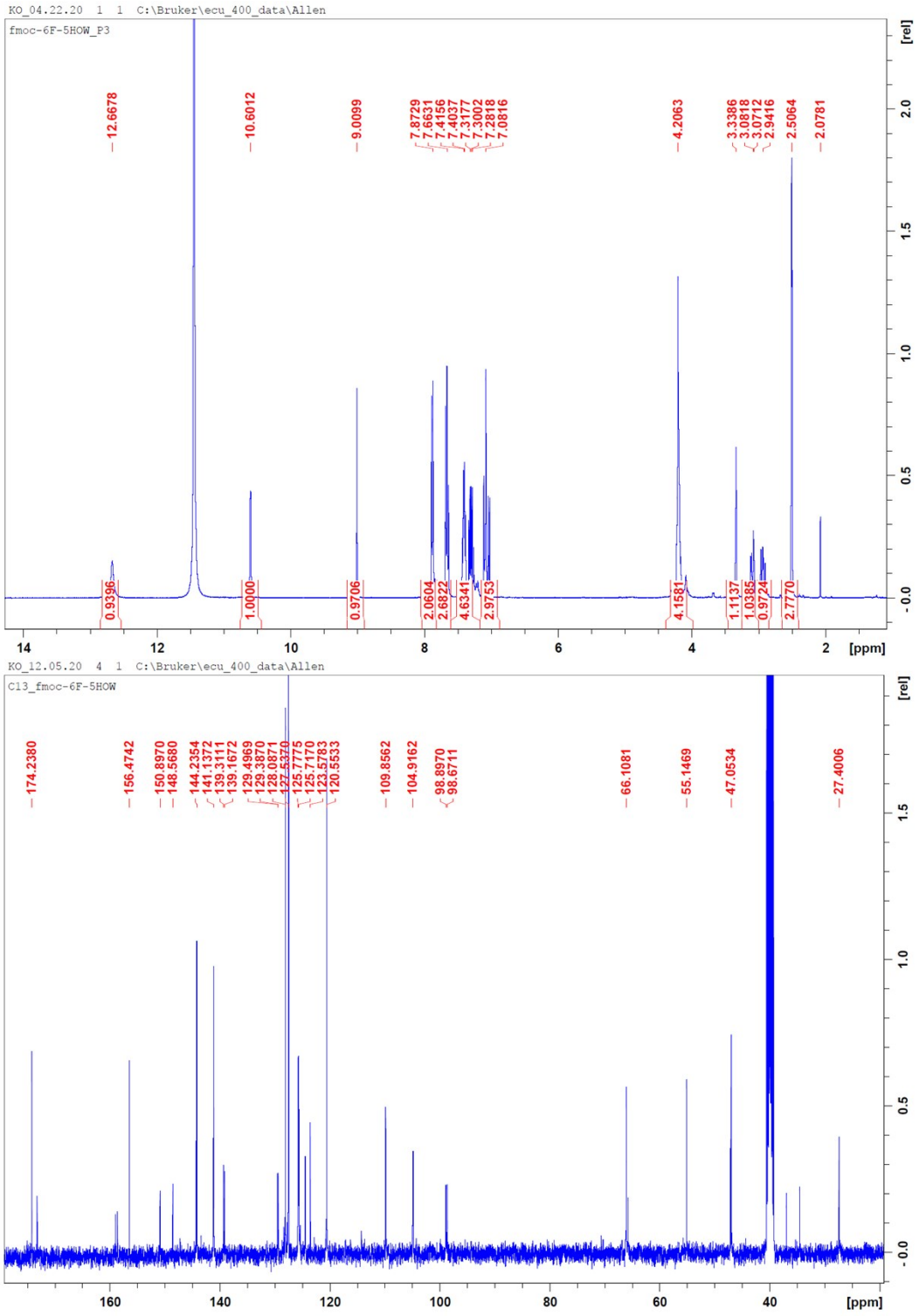


Figure S9. ^{1}H (top) and ^{13}C (bottom) NMR spectra of Fmoc-6F-5HOW in DMSO-d_6. The peak at ~11.5 ppm (top) is attributed to residual TFA.

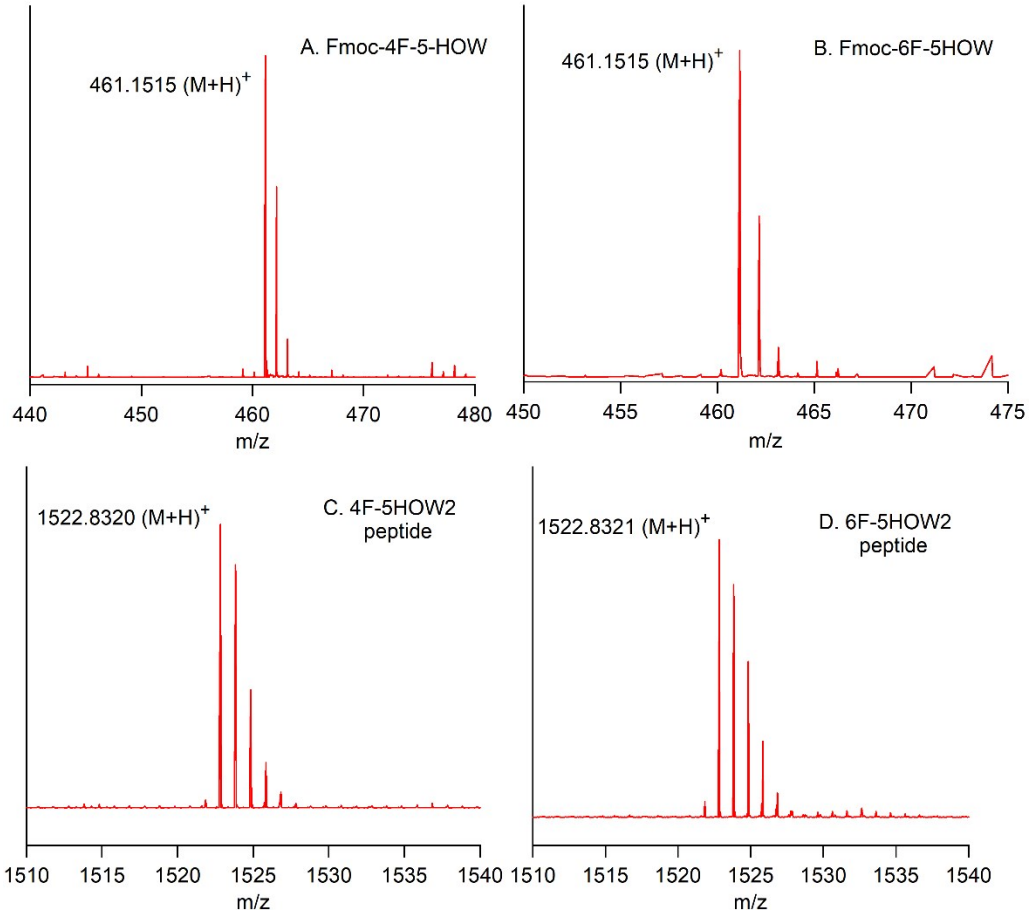


Figure S10. HRMS data collected (positive ion mode) using Shimadzu ESI-QToF of Fmoc-4F-5HOW (A) and Fmoc-6F-5HOW (B) and the peptides containing 4F-5HOW (C) and 6F-5HOW (D), collected in water/acetonitrile. The expected masses $(M+H)^+$ are (A,B) 461.1507 Da and (C,D) 1522.8289 Da.

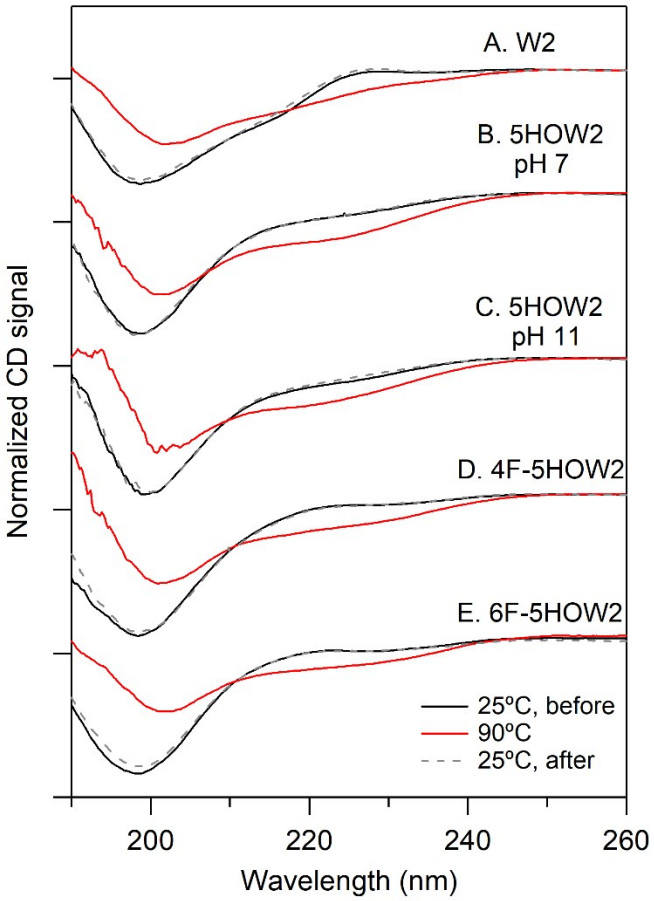


Figure S11. CD spectra of peptides W2 (A), 5HOW2 (B,C), 4F-5HOW2 (D), and 6F-5HOW2 (E). The spectra were collected at 25 °C before (black line) and after (gray dashed line) a 90 °C (red line) melt experiment. Solutions were 100 mM peptides in 25 mM sodium phosphate, pH 7.5 (A,B,D,E) and 25 mM sodium borate, pH 11 (C).

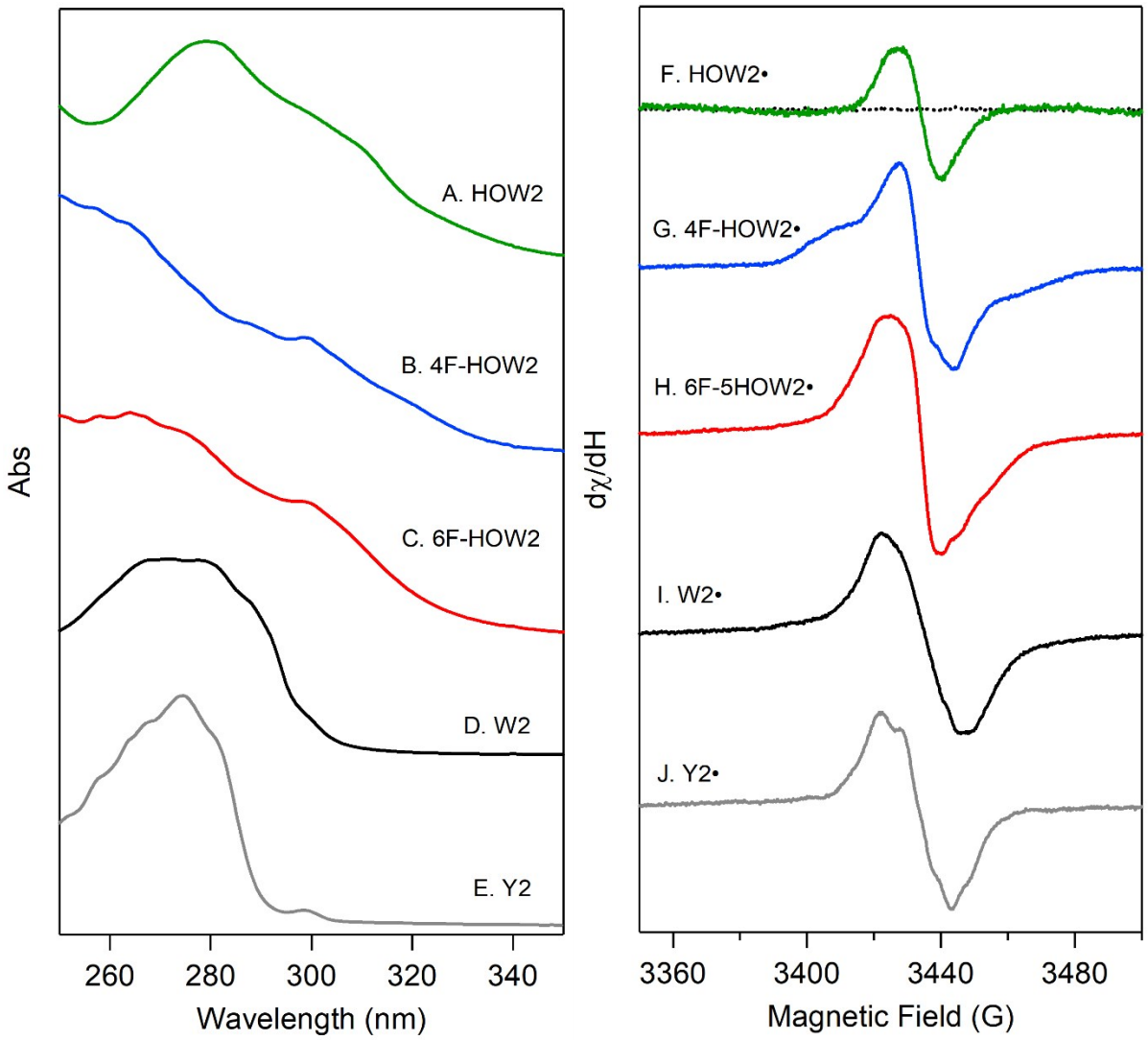


Figure S12. UV-visible (left) and EPR (right) spectra of (A,F) 5HOW2, (B,G) 4F-5HOW2, (C,H) 6F-5HOW2, (D,I) W2, and (E,J) Y2 β-hairpin peptides. The dashed line in (F) represents a spectrum of the sample before illumination.

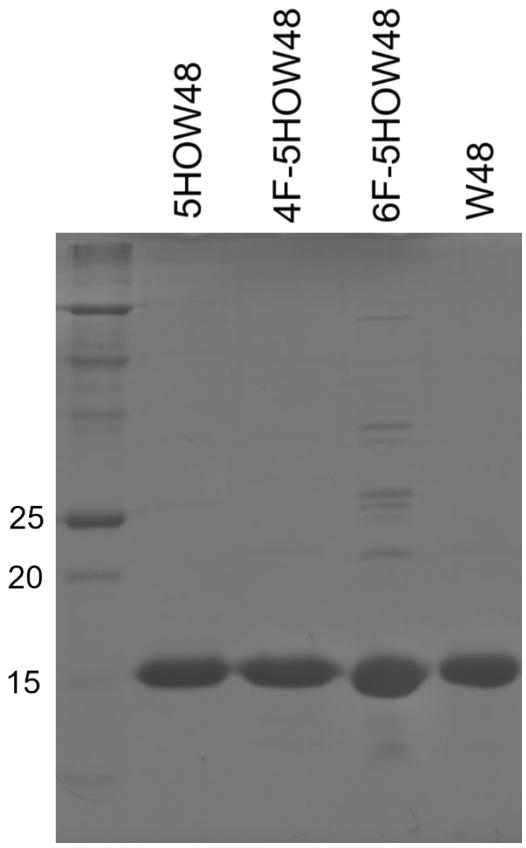


Figure S13. SDS-PAGE of azurin variants.

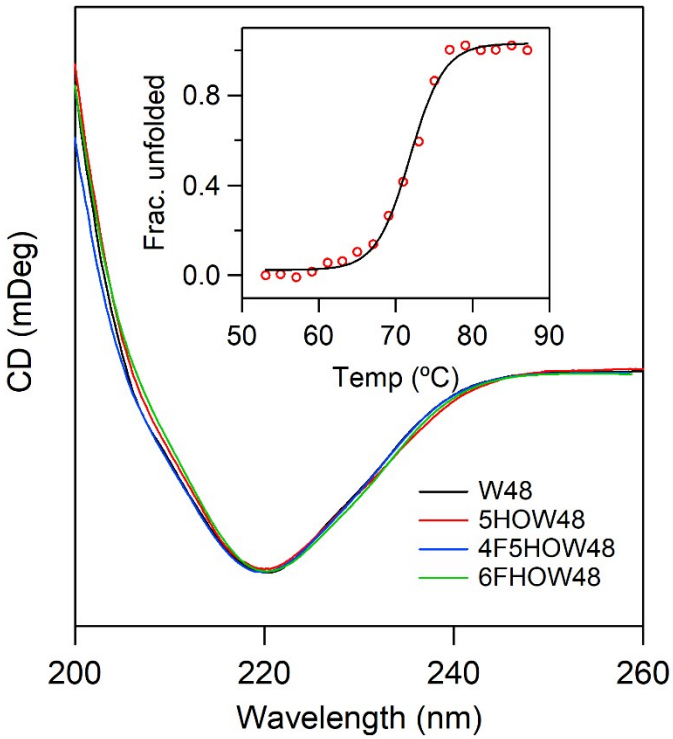


Figure S14. CD spectra of F_n-5HOW containing azurin in 25 mM potassium phosphate, pH 7.5. The inset shows a representative melt curve for azurin. The melting temperature, T_m, values are listed in **Table S4**.

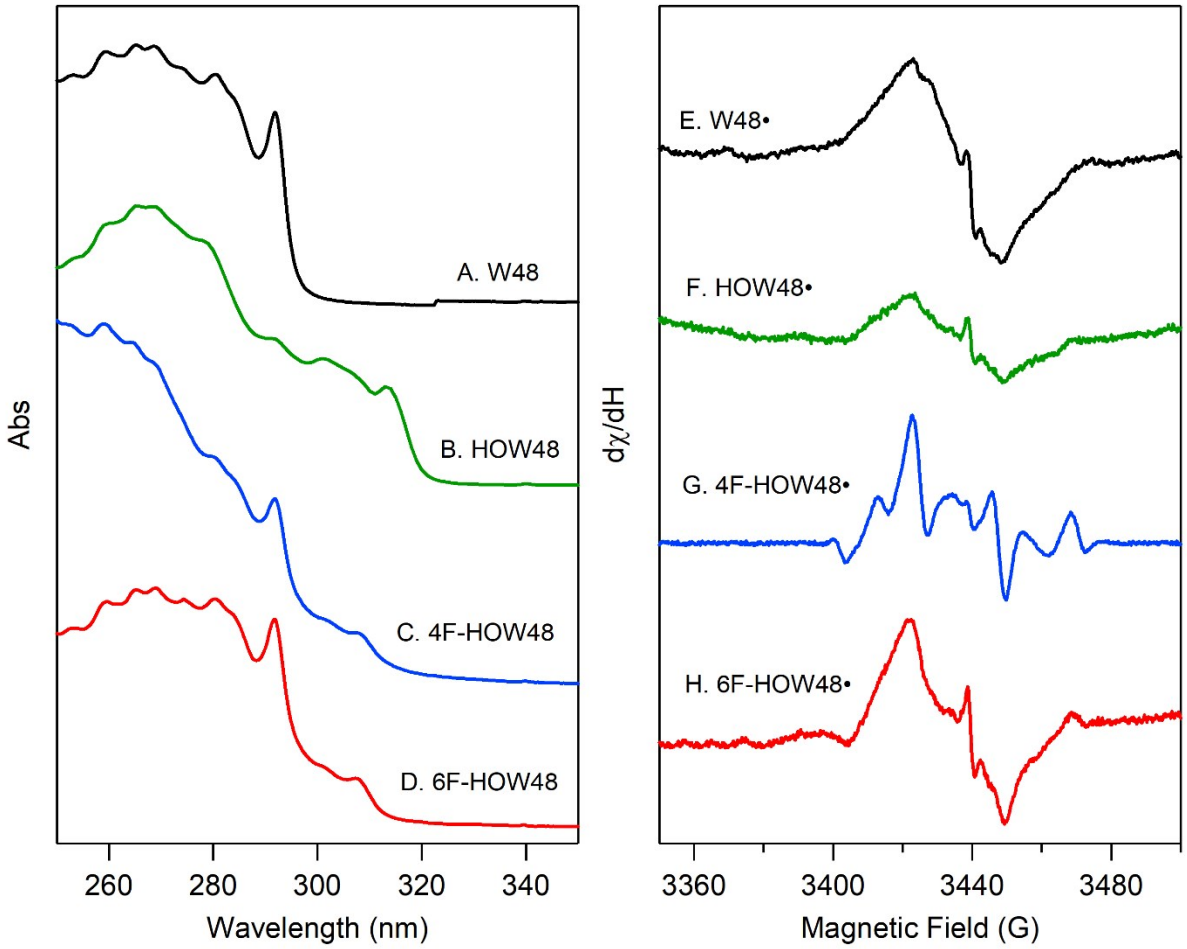


Figure S15. UV-visible (left) and EPR (right) spectra of azurin with incorporated F_n-5HOW.

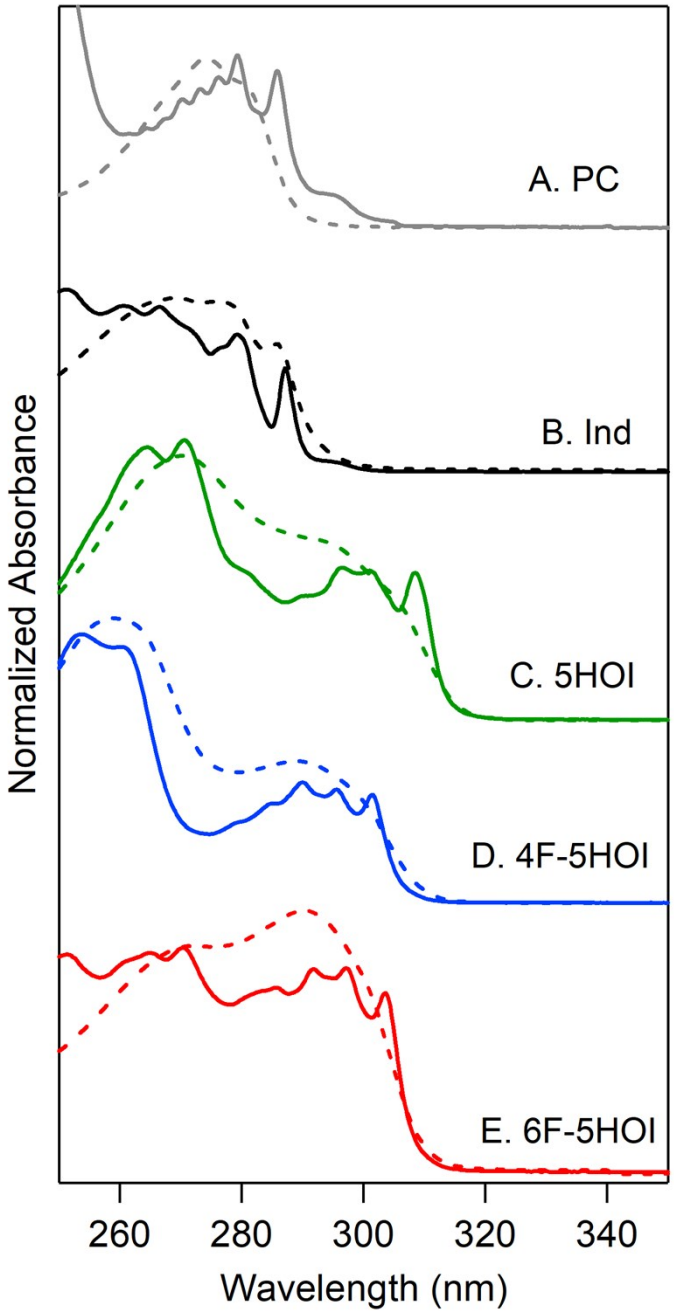


Figure S16. UV-visible absorbance of *p*-cresol (PC), indole (ind), and F_n-5HOIs in cyclohexane (ε = 2; solid lines) and in aqueous solution (ε = 80; dashed lines).

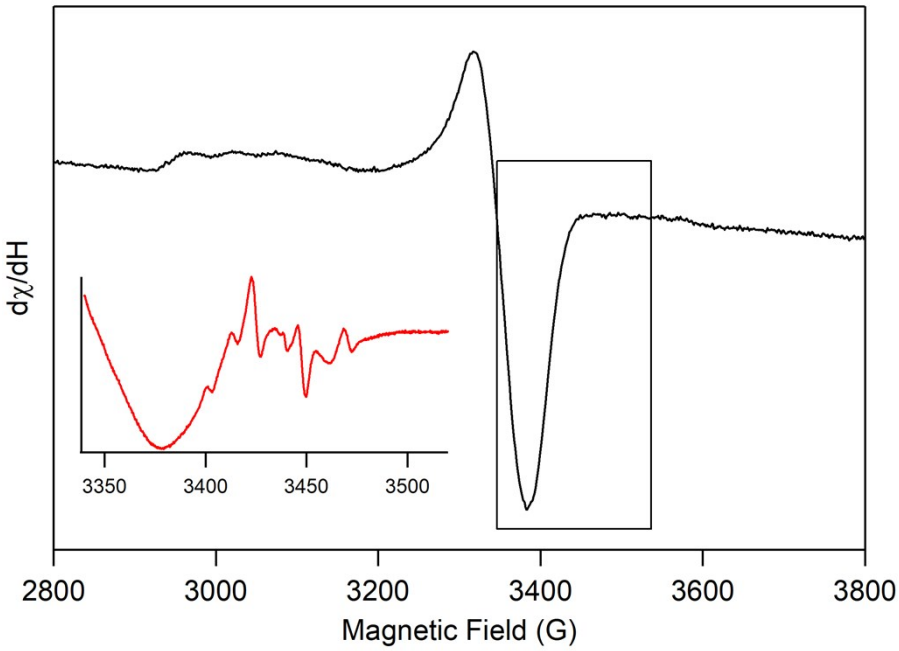


Figure S17. EPR spectrum of Cu^{2+} in azurin before photo-oxidation. *Inset*: focused view (from box) of EPR spectrum of photolysis-induced 4F-5HOW48• prior to background subtraction.

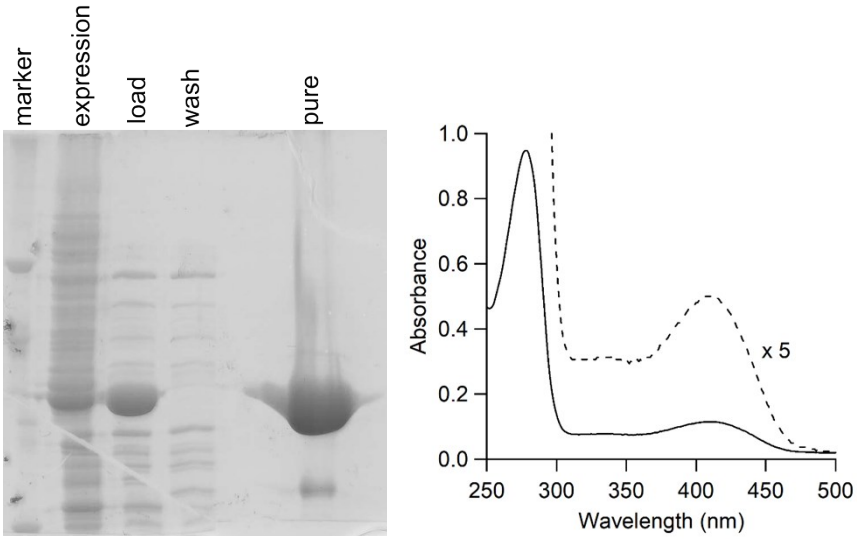


Figure S18. SDS-PAGE (left) and UV-vis (right) analysis of purified Tm9D8* TrpB synthase. The visible absorbance signal corresponding to the PLP cofactor, bound to TrpB, is represented by the dashed line.

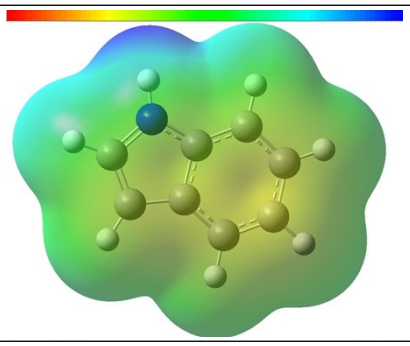
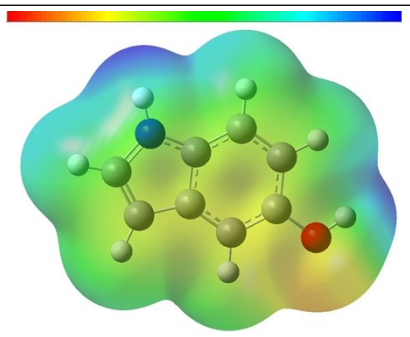
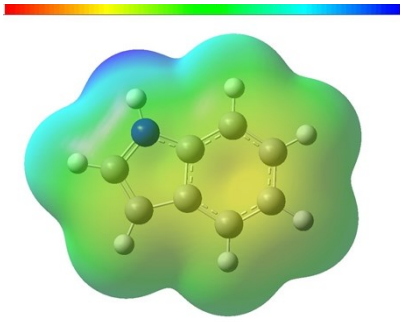
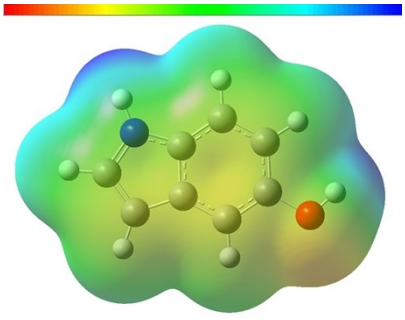
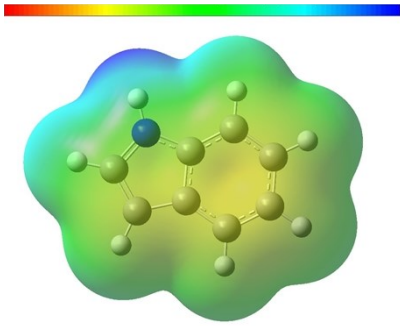
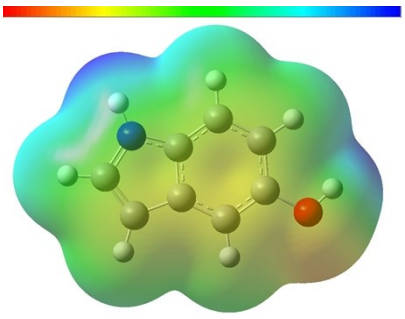
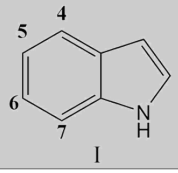
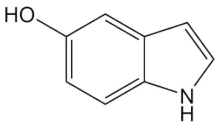
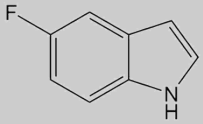
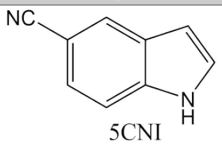
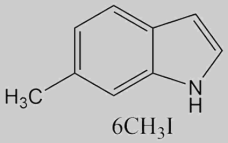
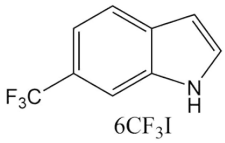
	Indole	5HOI
6-31+g*		
6-31++g(d,p)		
6-311++G(3df,2p)		
ccpvtz		

Figure S19. Electrostatic maps of indole and 5HOI for different basis sets.

Table S1. Reduction potentials (E°'), measured by SWV, of unnatural indoles at pH 7

	E°', mV at pH 7	ΔE (ref = I), mV
 I	983 ± 5	---
 5HOI	565 ± 3	- 418
 5FI	1076 ± 10[b]	+ 93
 5CNI	1052 ± 4	+ 69
 $6CH_3I$	N.D.[a]	N.D.[a]
 $6CF_3I$	996 ± 2	+ 13

[a]Not well defined; broad electrochemical response.

[b]5-fluoroindole was chosen as it was predicted to have the largest change from indole based on previously reported ionization potentials[13] and is consistent with the DFT calculated HOMO, reported herein (see **Figure 1A**).

Table S2. Electrochemical data and pK_a values for F_n-5HOIs

	pK_a[a]	*E* vs NHE (pH 7)[b]	IPs (eV)[c]	
			No solvent	With solvent
Trp, W	17[d]	983±5	7.51	5.61
Tyr, Y	10.3±0.01	928±4	N.D.	N.D.
5HOI	10.90±0.02	565±5	7.30	5.50
4F-5HOI	9.79±0.01	579±6	7.40	5.59
6F-5HOI	9.77±0.02	595±4	7.56	5.70

[a]The pK_a values were determined by UV-visible titrations; see **Figure S3**.

[b]Potentials were determined by SWV; see Figure **S2**.

[c]The IP values were calculated in two separate environments, no approximated solvent and pcm solvation model using the dielectric of water.

[d]Estimated from reference [19].

Table S3. Mass spectral assignments of peptic digest of 5HOW48 azurin

Sequence	Modifications	Charge	MH+ [Da]	m/z [Da]
wVL	W1(5HOW)	1	433.2446	433.2446
ITVD		1	447.2449	447.2449
FTVN		1	480.2452	480.2452
ADMQG		1	521.2023	521.2023
IQGND		1	546.2518	546.2518
QMQF		1	553.2438	553.2438
KPDDS		1	561.2513	561.2513
DVSKL		1	561.3250	561.3250
AHTKL		1	569.3405	569.3405
PGHSAL		1	581.3041	581.3041
AADMQG		1	592.2393	592.2393
STAADM		1	595.2389	595.2389
WVLST		1	605.3293	605.3293
YMFF		1	607.2584	607.2584
VVTDGM		1	621.2911	621.2911
wVLST	W1(5HOW)	1	621.3242	621.3242
AECSVD		1	623.2339	623.2339
GTLTLK		1	632.3975	632.3975
NTNAIT		1	633.3201	633.3201
LKPDDS		1	674.3353	674.3353
WVLSTA		1	676.3665	676.3665
IAHTKL		1	682.4246	682.4246
PDDSRV		1	688.3259	688.3259
wVLSTA	W1(5HOW)	1	692.3613	692.3613
QFNTNA		1	694.3152	694.3152
ASGLDKD		1	705.3413	705.3413
FDVSKL		1	708.3926	708.3926
WVLSTAA		1	747.4035	747.4035
VDIQGND		1	760.3470	760.3470
KGTLTLK		2	760.4927	380.7500
wVLSTAA	W1(5HOW)	1	763.3984	763.3984
STAADMQG		1	780.3192	780.3192
IQGNDQM		1	805.3506	805.3506
QGVVTDGM		1	806.3712	806.3712
KPDDSRV		2	816.4211	408.7142
MQFNTNA		1	825.3560	825.3560
TFPGHSAL		1	829.4203	829.4203
MKGTLTLK		2	891.5333	446.2703
SKLKEGEQ		2	918.4890	459.7481
LKPDDSRV		2	929.5049	465.2561

RVIAHTKL		1	937.5942	937.5942
VVTDGMASGL		1	949.4659	949.4659
QMQFNTNA		1	953.4146	953.4146
IGSGEKDSVT		2	992.4893	496.7483
VSKLKEGEQ		2	1017.5573	509.2823
ITVDKSCKQ		2	1021.5343	511.2708
SRVIAHTKL		2	1024.6260	512.8167
FCTFPGHSAL		2	1079.4982	540.2527
IQGNDQMQF		2	1080.4778	540.7426
TDGMASGLDKD		1	1109.4779	1109.4779
DVSKLKEGEQ		2	1132.5840	566.7957
IGSGEKDSVTF		2	1139.5582	570.2827
FFCTFPGHSAL		2	1226.5662	613.7867
IGSGEKDSVTFD		2	1254.5849	627.7961
VDKSCKQFTVN		2	1268.6299	634.8186
PGNLPKNVMGHN		2	1277.6412	639.3242
FDVSKLKEGEQ		2	1279.6532	640.3303
VVTDGMASGLDKD		2	1307.6150	654.3112
TVDKSCKQFTVN		2	1369.6781	685.3427
TVDKSCKQFTVNL		3	1482.7621	494.9256
ITVDKSCKQFTVN		2	1482.7625	741.8849
QGVVTDGMASGLDKD		2	1492.6950	746.8511
SHPGNLPKNVMGHN		2	1501.7328	751.3701
LSHPGNLPKNVMGHN		2	1614.8173	807.9123
DMQGVVTDGMASGLDKD		2	1738.7630	869.8851
ADMQGVVTDGMASGLDKD		2	1809.7995	905.4034
AADMQGVVTDGMASGLDKD		2	1880.8368	940.9221
IGSGEKDSVTFDVSKLKEGEQ		3	2253.1243	751.7130

Table S4. CD-derived T_m values for F_n-5HOW in azurin

Azurin	T_m (°C)
W48 (natural W)	74.0 ± 0.7
5HOW48	71.9 ± 0.2
4F-5HOW48	61.6 ± 0.6
6F-5HOW48	71.5 ± 0.5

Table S5. Electrochemical (SWV) potentials (in mV) collected at pH 7.

	E°, AA	E°, azurin	E°, peptide
Tyr	928 ± 4	N.D.	880 ± 20
Trp	983 ± 5	952 ± 6	989 ± 9
5HOW	565 ± 5	620 ± 10	568 ± 12
4F-5HOW	579 ± 6	632 ± 12	576 ± 5
6F-5HOW	595 ± 4	656 ± 7	590 ± 3

Table S6. Cartesian coordinates, energies (in Hartrees) of all complexes reported herein in standard xyz format. The xyz format lists the total number of atoms on the first line, a descriptive title on the second line that contains: the charge and multiplicity, structure descriptor and the total energy in Hartrees. The subsequent lines list the atom by atomic number followed by the Cartesian coordinates (in angstroms).

Solvent Calculations

Indole Ring

16

Neutral singlet indole -363.839546867

1	-0.552026	-1.428045	-1.727001
6	-1.350653	-1.249711	-1.018718
6	-1.225682	-0.620270	0.197451
6	-2.734023	-1.618940	-1.160637
1	-0.354728	-0.194518	0.676968
7	-2.455446	-0.575634	0.824573
6	-3.402004	-1.178439	0.020801
1	-2.629631	-0.164955	1.731246
6	-3.482384	-2.274539	-2.159146
6	-4.775232	-1.375347	0.222862
1	-5.266956	-1.032358	1.129568
6	-5.486431	-2.026755	-0.782381
1	-6.552969	-2.195524	-0.657431
6	-4.846207	-2.472296	-1.962551
1	-2.999506	-2.620774	-3.070127
1	-5.432386	-2.976980	-2.726511

16

Cationic doublet indole -363.633534396

1	-0.578039	-1.435934	-1.732367
6	-1.383644	-1.263627	-1.031942
6	-1.247663	-0.609224	0.229958
6	-2.740138	-1.620541	-1.161179
1	-0.362114	-0.190731	0.688218
7	-2.434376	-0.567225	0.832859
6	-3.407481	-1.179939	0.020304
1	-2.608150	-0.153565	1.743576
6	-3.481732	-2.280281	-2.170974
6	-4.752800	-1.366814	0.230549
1	-5.254450	-1.028763	1.131101
6	-5.473772	-2.032358	-0.798899
1	-6.538196	-2.194905	-0.662680
6	-4.851389	-2.477589	-1.970781
1	-2.993143	-2.622855	-3.077525
1	-5.439176	-2.980733	-2.731251

5-hydroxyindole solvent

17

Neutral singlet 5-HOI -439.062412021

1	-0.452051	0.925161	-0.145235
6	-1.250836	1.141341	0.552169
6	-1.134371	1.873570	1.712566
6	-2.623783	0.726758	0.453003
1	-0.270103	2.359167	2.145200
7	-2.357043	1.940346	2.344676
6	-3.294222	1.247159	1.598555
1	-2.536047	2.423578	3.213643
6	-3.351430	-0.029799	-0.487384
6	-4.658522	1.028603	1.817809
1	-5.164823	1.427957	2.692437
6	-5.359375	0.278054	0.876133
1	-6.421423	0.090530	1.018845
6	-4.706447	-0.243957	-0.264102
1	-2.875897	-0.443302	-1.372768
8	-5.394970	-0.990525	-1.203310
1	-6.325793	-1.072864	-0.940323

17

Cationic doublet 5-HOI -438.860765078

1	-0.433497	0.925713	-0.153477
6	-1.230373	1.141365	0.543924
6	-1.129492	1.865487	1.698100
6	-2.615743	0.719698	0.436456
1	-0.282960	2.358873	2.152320
7	-2.379154	1.916238	2.315010
6	-3.295412	1.240453	1.588033
1	-2.563750	2.398403	3.189047
6	-3.325298	-0.018800	-0.483028
6	-4.676378	1.028628	1.823289
1	-5.162498	1.437084	2.703236

6	-5.385860	0.287424	0.900137
1	-6.445684	0.099144	1.039994
6	-4.725554	-0.234601	-0.241077
1	-2.876682	-0.442451	-1.375576
8	-5.351540	-0.959625	-1.166374
1	-6.297260	-1.081257	-0.958100

4-Fluoro-5-hydroxyindole solvent

17

Neutral singlet 4F5-HOI -538.300363174

1	-0.457526	0.924179	-0.141469
6	-1.252371	1.143126	0.557668
6	-1.137191	1.875807	1.717779
6	-2.625480	0.735125	0.468397
1	-0.271761	2.360433	2.148559
7	-2.358592	1.944376	2.350605
6	-3.300754	1.253644	1.611165
1	-2.535748	2.428864	3.219487
6	-3.377614	-0.011557	-0.444880
6	-4.665966	1.037452	1.833552
1	-5.170036	1.438059	2.707907
6	-5.367838	0.287331	0.893811
1	-6.429581	0.099627	1.035889
6	-4.732112	-0.243191	-0.251565
9	-2.765226	-0.526309	-1.554176
8	-5.398245	-0.988509	-1.197534
1	-6.331094	-1.076680	-0.943282

17

Cationic doublet 4F5-HOI -538.095235317

1	-0.438429	0.930604	-0.143379
6	-1.236290	1.144786	0.552534
6	-1.135315	1.876170	1.718770
6	-2.612226	0.731738	0.456231
1	-0.282028	2.366027	2.164140
7	-2.366858	1.926990	2.326786
6	-3.295274	1.245361	1.595177
1	-2.552887	2.408946	3.200964
6	-3.353175	-0.005728	-0.446695
6	-4.667032	1.036725	1.830331
1	-5.153626	1.445441	2.709634
6	-5.392876	0.289710	0.905035
1	-6.452116	0.109952	1.057690
6	-4.762713	-0.238050	-0.230713
9	-2.804046	-0.525773	-1.538048
8	-5.362794	-0.965737	-1.171343
1	-6.309450	-1.095384	-0.975203

6-Fluoro-5-hydroxyindole solvent

17

Neutral singlet 6F5-HOI -538.301490237

1	-0.435694	0.929897	-0.145052
6	-1.234286	1.145648	0.552477
6	-1.119813	1.876600	1.711989
6	-2.607225	0.730061	0.450833
1	-0.257828	2.363067	2.147591
7	-2.345902	1.942037	2.342987
6	-3.278164	1.250264	1.596795
1	-2.526671	2.422887	3.212993
6	-3.330610	-0.027190	-0.492485
6	-4.644555	1.035138	1.821417
1	-5.175027	1.422197	2.685539
6	-5.302988	0.287588	0.865732
9	-6.646339	0.030709	1.010995
6	-4.684892	-0.249191	-0.282557
1	-2.853277	-0.440027	-1.376801
8	-5.404856	-0.986380	-1.192960
1	-6.329009	-1.051528	-0.897580

17

Cationic doublet 6F5-HOI -538.092556535

1	-0.417712	0.930104	-0.155319
6	-1.213643	1.145488	0.542862
6	-1.115938	1.865225	1.692033
6	-2.600011	0.719845	0.430492
1	-0.273494	2.360816	2.151293
7	-2.375472	1.915280	2.313820
6	-3.284516	1.243615	1.589596
1	-2.560300	2.396831	3.188126
6	-3.299843	-0.019205	-0.493371
6	-4.668666	1.037484	1.834177
1	-5.175909	1.435898	2.705894
6	-5.332313	0.300566	0.897487
9	-6.652128	0.033433	1.012961
6	-4.691982	-0.239277	-0.262231
1	-2.846425	-0.440675	-1.384186
8	-5.363509	-0.954411	-1.150572

1 -6.305276 -1.049243 -0.901149

NO SOLVENT

Indole ring

16

Neutral singlet indole -363.831837620

1	-0.555176	-1.428605	-1.726375
6	-1.352454	-1.249325	-1.017322
6	-1.223759	-0.620967	0.195515
6	-2.735574	-1.617945	-1.158445
1	-0.350297	-0.196201	0.671437
7	-2.455568	-0.575002	0.824923
6	-3.403556	-1.178732	0.020225
1	-2.631163	-0.166520	1.730365
6	-3.482353	-2.273042	-2.156126
6	-4.775361	-1.375721	0.221627
1	-5.270773	-1.034226	1.127568
6	-5.485309	-2.026721	-0.782973
1	-6.551751	-2.195853	-0.658643
6	-4.844693	-2.471190	-1.961075
1	-2.998479	-2.618998	-3.066447
1	-5.429998	-2.976039	-2.725286

16

Cationic doublet indole -363.556328747

1	-0.577337	-1.435787	-1.732003
6	-1.383029	-1.262419	-1.030417
6	-1.243096	-0.609296	0.227996
6	-2.739110	-1.618317	-1.158001
1	-0.354331	-0.191920	0.683946
7	-2.433528	-0.565106	0.835823
6	-3.407861	-1.178075	0.023189
1	-2.605010	-0.151547	1.746660
6	-3.482954	-2.279915	-2.169852
6	-4.753801	-1.366651	0.230576
1	-5.263084	-1.031796	1.129382
6	-5.475562	-2.033756	-0.801238
1	-6.540373	-2.196769	-0.664877
6	-4.852688	-2.478321	-1.971913
1	-2.995102	-2.623406	-3.077243
1	-5.439398	-2.982006	-2.733060

5-hydroxyindole no solvent

17

Neutral singlet 5-HOI -439.050329156

1	-0.456790	0.923566	-0.144899
6	-1.253652	1.141159	0.553629
6	-1.133162	1.872689	1.709826
6	-2.626730	0.727163	0.455312
1	-0.266130	2.356871	2.138652
7	-2.357647	1.941412	2.345383
6	-3.296529	1.247020	1.599249
1	-2.537067	2.422212	3.213465
6	-3.351227	-0.028210	-0.484607
6	-4.658950	1.028608	1.818190
1	-5.168795	1.426569	2.692123
6	-5.357272	0.278156	0.875425
1	-6.420141	0.091218	1.018732
6	-4.705653	-0.243727	-0.264182
1	-2.879067	-0.442812	-1.370365
8	-5.389653	-0.988669	-1.203568
1	-6.318672	-1.071447	-0.940451

17

Cationic doublet 5-HOI -438.782703583

1	-0.433141	0.925457	-0.151185
6	-1.231871	1.141147	0.544968
6	-1.126863	1.868183	1.698855
6	-2.617148	0.720328	0.440269
1	-0.275081	2.359951	2.146879
7	-2.374602	1.920000	2.316887
6	-3.296529	1.241287	1.589826
1	-2.556713	2.403395	3.190620
6	-3.328118	-0.019442	-0.479719
6	-4.675485	1.028645	1.825419
1	-5.166627	1.434784	2.705016
6	-5.388252	0.286233	0.902364
1	-6.448618	0.101112	1.047932
6	-4.731154	-0.235405	-0.239171
1	-2.884287	-0.446210	-1.374016
8	-5.347138	-0.957916	-1.169994
1	-6.295510	-1.089772	-0.983038

4-fluoro-5-hydroxyindole no solvent

17
Neutral singlet 4F5-HOI -538.286413173

1	-0.467913	0.919093	-0.145087
6	-1.256809	1.142329	0.558697
6	-1.135678	1.874368	1.714117
6	-2.630477	0.735707	0.471946
1	-0.267133	2.357424	2.140663
7	-2.359267	1.945637	2.352248
6	-3.303962	1.253738	1.613073
1	-2.536062	2.428373	3.219923
6	-3.373660	-0.012205	-0.447353
6	-4.667392	1.037558	1.834915
1	-5.176641	1.435842	2.708032
6	-5.364284	0.287108	0.891589
1	-6.427084	0.099767	1.033089
6	-4.729658	-0.243108	-0.252999
9	-2.759466	-0.519583	-1.544858
8	-5.395882	-0.986369	-1.197282
1	-6.325769	-1.073902	-0.938798

17
Cationic doublet 4F5-HOI -538.015107010

1	-0.444012	0.925076	-0.144839
6	-1.240622	1.142759	0.552634
6	-1.133147	1.878208	1.718631
6	-2.614862	0.733573	0.461965
1	-0.273035	2.365572	2.156049
7	-2.361907	1.932315	2.330393
6	-3.296997	1.247266	1.598959
1	-2.544004	2.416200	3.203764
6	-3.353736	-0.007952	-0.448189
6	-4.667265	1.036551	1.833162
1	-5.160692	1.442171	2.711526
6	-5.393879	0.288253	0.906948
1	-6.453754	0.110756	1.064197
6	-4.767124	-0.240122	-0.231162
9	-2.803148	-0.521327	-1.528964
8	-5.360091	-0.964585	-1.174433
1	-6.308862	-1.102936	-0.998726

<u>6-fluoro-5-hydroxyindole no solvent</u>

17
Neutral singlet 6F5-HOI -538.291222227

1	-0.438387	0.928866	-0.143531
6	-1.235852	1.145750	0.554428
6	-1.117652	1.876611	1.709785
6	-2.608858	0.730349	0.452967
1	-0.253160	2.361834	2.141873
7	-2.346188	1.943791	2.343019
6	-3.279932	1.249955	1.596401
1	-2.527765	2.421709	3.212328
6	-3.329626	-0.026422	-0.490568
6	-4.645373	1.034050	1.820309
1	-5.182419	1.418022	2.682040
6	-5.302720	0.285951	0.864489
9	-6.643837	0.026961	1.007624
6	-4.683440	-0.249512	-0.283152
1	-2.854662	-0.440008	-1.375180
8	-5.402672	-0.984223	-1.190584
1	-6.324590	-1.041907	-0.890336

17
Cationic doublet 6F5-HOI -538.013838280

1	-0.414488	0.928832	-0.151703
6	-1.212896	1.145081	0.544257
6	-1.112772	1.866068	1.690185
6	-2.599510	0.719436	0.432134
1	-0.266157	2.360278	2.145123
7	-2.375450	1.919264	2.314926
6	-3.286952	1.245528	1.591562
1	-2.558579	2.402765	3.188175
6	-3.298922	-0.020705	-0.492573
6	-4.671717	1.037896	1.836740
1	-5.187297	1.432635	2.706491
6	-5.335390	0.299692	0.900756
9	-6.647834	0.027682	1.009856
6	-4.691782	-0.239679	-0.262215
1	-2.847419	-0.444851	-1.384526
8	-5.362968	-0.950406	-1.149492
1	-6.307004	-1.047738	-0.907782

Indole radical
15
Neutral doublet indole radical -363.181107259

1	-0.554678	-1.428930	-1.728788
6	-1.369357	-1.260733	-1.033553
6	-1.289629	-0.609642	0.247112
6	-2.739706	-1.632593	-1.186492
1	-0.390991	-0.194856	0.692971
7	-2.449854	-0.549431	0.874784
6	-3.376968	-1.178198	0.009626
6	-3.481487	-2.284250	-2.178656
6	-4.728456	-1.368080	0.216329
1	-5.207278	-1.020549	1.127339
6	-5.469457	-2.028654	-0.794162
1	-6.534941	-2.187907	-0.650498
6	-4.858566	-2.477517	-1.967522
1	-3.011977	-2.636061	-3.094213
1	-5.451757	-2.981166	-2.725673

5-hydroxyindole radical
16
Neutral doublet 5HOI• -438.420429850

1	-0.431184	0.932469	-0.140652
6	-1.233152	1.145912	0.552750
6	-1.128478	1.872047	1.706650
6	-2.609887	0.722347	0.439186
1	-0.274044	2.362277	2.152099
7	-2.372705	1.927973	2.328982
6	-3.292994	1.239725	1.584667
1	-2.563860	2.405615	3.197981
6	-3.320554	-0.025595	-0.493609
6	-4.673789	1.024599	1.817418
1	-5.159958	1.433841	2.700331
6	-5.372624	0.286887	0.896543
1	-6.432805	0.087067	1.018000
6	-4.734776	-0.271305	-0.297194
1	-2.856029	-0.443771	-1.381990
8	-5.401626	-0.946862	-1.128797

4-fluoro-5-hydroxyindole radical
16
Neutral doublet 4F-5HOI• -537.659072808

1	-0.440144	0.930695	-0.140366
6	-1.235390	1.148529	0.557767
6	-1.130195	1.875227	1.712790
6	-2.611000	0.732285	0.456024
1	-0.274360	2.364451	2.156226
7	-2.372408	1.932033	2.335482
6	-3.297306	1.244931	1.594785
1	-2.562439	2.409841	3.204718
6	-3.344154	-0.011665	-0.460239
6	-4.679899	1.030498	1.829481
1	-5.163809	1.440506	2.712584
6	-5.381894	0.293609	0.911608
1	-6.441907	0.094893	1.034873
6	-4.763346	-0.272611	-0.286834
9	-2.737991	-0.512166	-1.544711
8	-5.414729	-0.946852	-1.122801

6-fluoro-5-hydroxyindole radical

16
Neutral doublet 6F-5HOI• -537.657784968

1	-0.414956	0.937954	-0.139329
6	-1.217859	1.150308	0.553099
6	-1.117057	1.874641	1.706042
6	-2.594880	0.723762	0.435174
1	-0.265385	2.366203	2.154984
7	-2.365407	1.928968	2.327320
6	-3.281226	1.240954	1.581430
1	-2.558930	2.405679	3.196236
6	-3.299001	-0.023927	-0.499472
6	-4.659336	1.027975	1.816789
1	-5.174232	1.422658	2.687803
6	-5.331846	0.289012	0.883110
9	-6.644283	0.052693	1.051954
6	-4.711734	-0.281303	-0.322769
1	-2.830907	-0.440385	-1.386453
8	-5.385513	-0.951908	-1.143187

Tryptophan radical
26
Neutral doublet W• -685.738973

6	0.409140	-0.308930	0.186114
6	1.555252	-0.469318	1.058329
6	0.969060	0.054880	-1.085195
1	1.502283	-0.739456	2.106165
7	2.707285	-0.244712	0.457104
6	2.380467	0.082476	-0.878539
6	0.434437	0.355483	-2.343880
6	3.248332	0.403370	-1.905509
1	4.321778	0.421429	-1.739108
6	2.698317	0.705501	-3.171628
1	3.362912	0.960720	-3.992991
6	1.316185	0.682626	-3.386919
1	-0.638890	0.343610	-2.521074
6	-1.040386	-0.507987	0.496880
1	-1.269989	-1.583724	0.552947
1	-1.641853	-0.112719	-0.330959
6	-1.513568	0.144309	1.823386
1	-1.128250	1.166496	1.875726
7	-0.998145	-0.598794	2.972145
1	-1.168410	-0.096484	3.841145
1	-1.452845	-1.507278	3.050417
6	-3.038700	0.281656	1.794573
8	-3.661982	-0.917089	1.954031
1	-4.623410	-0.755071	1.914356
8	-3.649496	1.317742	1.642803
1	0.920477	0.921264	-4.370319

5-hydroxytryptophan radical
27
Neutral doublet 5-HOW• -760.976231

6	0.278539	-0.243244	0.197901
6	1.327248	-0.375338	1.071371
6	0.860800	0.073538	-1.095121
1	1.312057	-0.611088	2.124833
7	2.526595	-0.152678	0.398437
6	2.278557	0.119918	-0.917400
1	3.440513	-0.186389	0.826455
6	0.330089	0.318158	-2.356634
6	3.176535	0.408758	-1.973913
1	4.249899	0.437107	-1.798436
6	2.651287	0.651257	-3.217299
1	3.287004	0.877977	-4.067630
6	1.209829	0.616022	-3.469553
1	-0.736882	0.297432	-2.559904
8	0.749684	0.836605	-4.623479
6	-1.193926	-0.398377	0.467909
1	-1.499625	-1.452168	0.393377
1	-1.748644	0.129261	-0.318196
6	-1.658203	0.149114	1.836639
1	-1.278347	1.167606	1.957822
7	-1.125567	-0.666469	2.933981
1	-1.353906	-0.250961	3.835740
1	-1.540540	-1.597209	2.919553
6	-3.183958	0.271945	1.847940
8	-3.794025	-0.944197	1.876121
1	-4.757247	-0.788018	1.874644
8	-3.807764	1.311437	1.834842

4-fluoro-5-hydrotryptophan radical
27
Neutral doublet 4F-5HOW• -860.214369

6	0.264836	-0.227706	0.185051
6	1.312663	-0.351433	1.063887
6	0.861991	0.075045	-1.102738
1	1.288073	-0.578033	2.119049
7	2.513012	-0.136590	0.396374
6	2.275921	0.126017	-0.923445
1	3.425241	-0.166740	0.828500
6	0.377360	0.312371	-2.384259
6	3.188190	0.407784	-1.971847
1	4.258280	0.436822	-1.780487
6	2.683192	0.640491	-3.224038
1	3.330475	0.861489	-4.066953
6	1.250707	0.605320	-3.509730
8	0.788438	0.814104	-4.658865
6	-1.208835	-0.378402	0.466139
1	-1.515709	-1.430691	0.377798
1	-1.769822	0.160086	-0.304132
6	-1.650760	0.146136	1.850530
1	-1.269792	1.163024	1.981676
7	-1.103926	-0.684822	2.930356
1	-1.335660	-0.290316	3.840737
1	-1.509018	-1.619501	2.898262
6	-3.176177	0.269486	1.887462
8	-3.786494	-0.946542	1.890839
1	-4.749507	-0.790444	1.911892
8	-3.797892	1.309605	1.919607
9	-0.944787	0.273442	-2.621665

6-fluoro-5-hydroxytryptophan radical
27
Neutral doublet 6F-5HOW• -860.213643

6	0.271676	-0.240136	0.204526
6	1.317903	-0.372926	1.077715
6	0.855788	0.073265	-1.090102
1	1.302021	-0.607362	2.131335
7	2.521699	-0.154902	0.404333
6	2.275707	0.116450	-0.909710
1	3.434671	-0.185487	0.834458
6	0.325001	0.319821	-2.348681
6	3.179415	0.400329	-1.959525
1	4.255318	0.432501	-1.814062
6	2.633120	0.637788	-3.190440
6	1.190197	0.614454	-3.471123
1	-0.741939	0.302880	-2.551042
8	0.748899	0.839508	-4.624957
6	-1.202144	-0.390982	0.470745
1	-1.510837	-1.443330	0.388357
1	-1.754756	0.144823	-0.311204
6	-1.665883	0.148829	1.842999
1	-1.291154	1.169088	1.964878
7	-1.125680	-0.667162	2.935784
1	-1.357001	-0.257881	3.839580
1	-1.533961	-1.600766	2.917700
6	-3.192397	0.264468	1.853578
8	-3.796748	-0.953235	1.913075
1	-4.760785	-0.802012	1.907573
8	-3.820122	1.300706	1.813217
9	3.441993	0.911270	-4.229008

5-hydroxytryptophan deprotonated carboxylate radical
26
Anionic doublet 5-HOW•deprot carbox -760.442989

6	0.072423	-0.291781	0.237080
6	1.115371	-0.473793	1.108854
6	0.678545	0.057079	-1.041493
1	1.068148	-0.743241	2.153758
7	2.329501	-0.254190	0.450306
6	2.098908	0.068288	-0.855028
1	3.238066	-0.313575	0.885627
6	0.166071	0.354038	-2.298769
6	3.007194	0.370431	-1.896613
1	4.080854	0.372623	-1.713251
6	2.497002	0.660560	-3.138431
1	3.145019	0.898677	-3.977014
6	1.058685	0.661747	-3.401183
1	-0.901327	0.364885	-2.500208
8	0.613499	0.924324	-4.556050
6	-1.399364	-0.423951	0.526994
1	-1.704919	-1.478336	0.443942
1	-1.971033	0.122842	-0.231123
6	-1.852244	0.096753	1.909241
1	-1.519718	1.137623	2.003869
7	-1.236233	-0.700606	2.997147
1	-1.305523	-0.146720	3.852577
1	-1.916695	-1.453682	3.154983
6	-3.428603	0.075146	1.956818
8	-3.934699	-0.890014	2.595524
8	-3.998927	1.004873	1.332444

Acetylated 5-hydroxytryptophan radical
33
Neutral doublet Ac-5-HOW•-NH2 -893.778054

6	0.547281	-0.125651	-0.588309
6	1.517320	-0.080013	0.380989
6	1.239589	0.004539	-1.859655
1	1.444271	-0.161788	1.455532
7	2.767762	0.077099	-0.212220
6	2.632544	0.132017	-1.570888
1	3.639671	0.139150	0.293399
6	0.815722	0.029120	-3.183420
6	3.615411	0.285747	-2.579409
1	4.667480	0.381856	-2.320055
6	3.197370	0.306994	-3.885113
1	3.901274	0.419194	-4.703962
6	1.785532	0.178775	-4.250081
1	-0.228455	-0.059104	-3.469363
8	1.426900	0.196563	-5.459237
6	-0.942856	-0.292987	-0.457629
1	-1.231056	-1.329631	-0.698204
1	-1.443082	0.333724	-1.203338
6	-1.548612	0.083260	0.909194
1	-1.221634	1.099236	1.153472
7	-1.096457	-0.830037	1.955913
1	-0.769041	-1.747643	1.669026
6	-3.087208	0.171203	0.763723
8	-3.584926	0.989577	-0.000657
6	-0.819950	-0.539255	3.275887
8	-0.311081	-1.388836	4.001572
6	-1.165793	0.851998	3.776197
1	-0.498996	1.602592	3.333218
1	-1.030853	0.863714	4.858662
1	-2.194678	1.140151	3.534046
7	-3.817965	-0.691603	1.517242
1	-4.825664	-0.677333	1.431548
1	-3.379821	-1.362628	2.131921