A Water-Soluble Iridium Photocatalyst for Chemical Modification of Dehydroalanines in Peptides and Proteins

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S1 Organic Chemistry

S1.1 – General remarks
Chemicals were purchased from TCI Europe, Sigma-Aldrich, Acros, Strem Chemical, Handary, Chem-Impex, or Fluorochem. Solvents were purchased from Boom, Honeywell, VWR chemicals, Macron fine chemicals, or Sigma-Aldrich. All chemicals and solvents were used without further purification. Solvents used for reactions were either reagent-grade, HPLC-grade, or well dried with an MBraun-SPS-800. Solvents used for work-up and purification were of technical grade. Solvents were removed under reduced pressure at 40 °C (water bath), by lyophilisation on a Christ Alpha 2-4 LD plus, by drying under vacuum at room temperature over P2O5, or by an Eppendorf Concentrator plus. Reactions carried out under dry conditions were performed under N2- or Ar-atmosphere.

Column chromatography was performed by hand on silica gel (Silicycle SiliaFlash P60, 40-63 µm, 230-400 mesh or Merck silica gel 60, 40-63 µm) or automated on a Grace Reveletis Flash X1 Chromatography system. The crude products were loaded onto columns using either dry loading techniques with celite or liquid loading using the same eluent as used for running the column. Analytical thin-layer chromatography was carried out on pre-coated silica gel on aluminium sheets (Merck TLC Silica gel 60 / Kieselguhr F254). Compound spots were visualized under ultraviolet (UV) light (254 nm or 365 nm for fluorescent compounds), potassium permanganate (KMnO4) stain, p-anisaldehyde stain, or iodine on silica.

1H-, 19F-, and 13C-NMR spectra were recorded on a Varian Mercury Plus 400 MHz, Agilent Technologies 400 MHz/54 Premium Shielded, or Bruker Ascend 600 MHz at ambient temperature. 1H-NMR spectra are reported as follows: chemical shift (δ) in ppm (multiplicity, coupling constants J in Hz, number of protons). All chemical shifts (δ) are reported to the nearest 0.01 ppm relative to the residual solvent peak ((CD3)2SO = 2.50, D2O = 4.79, and CDCl3 = 7.26 ppm). The multiplicity and shape of the 1H-signals are designated by the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet, br = broad, or combinations thereof. NMR spectra: chemical shift (δ) are reported in ppm. All chemical shifts (δ) are reported to the nearest 0.1 ppm and referenced with respect to the residual solvent peak.

High-resolution mass spectrometry (HRMS) mass spectra of small organic molecules were recorded on a Thermo Fisher Scientific LTQ Orbitrap XL using either positive or negative ESI ionization method. All reported values were within 5 ppm of the calculated value. Elemental analyses were recorded on a EuroVector S.P.A. model Euro EA 3000 or on an Elementar vario MICRO cube. UV/vis absorption spectra were recorded on an AnalytikJena Speccord210 UV/vis spectrometer in 1 cm pathlength quartz cuvettes. Emission spectra were recorded on a Edinburgh Instruments FS-5 spectrofluorimeter in 1 cm pathlength quartz cuvettes. Photoredox catalysis was performed with a blue LED light set-up with 5 LED lights in a series circuit. LEDs were purchased from LED ENGIN (Mouser Electronics), serial number LZ1-10UA00-00U8. These LED lights have a wavelength of 407.5 nm, luminous flux / radiant flux of 550 mW (adjusted to 220 mW by using a 6.8 Ω resistor), and power rating of 5 W. Reaction mixtures were irradiated at ~ 6 distance.

Reaction mixtures were analysed by liquid-chromatography mass-spectrometry (LC-MS) performed on a Waters Acquity Ultra Performance LC with TQD mass detector (ESI+). Separation of methyl 2-acetamidoacrylate (8) reactions was achieved with a reversed-phase UPLC column (Acquity UPLC HSS T3, 1.8 µm, 2.1 mm x 150 mm) at 40 °C. UPLC grade 0.1% formic acid (FA) in H2O (solvent A) and 0.1% FA in acetonitrile (solvent B) were used as eluents. There was made use of a linear gradient: 90% A to 50% A over 8 min., then to 5% A over 1 min. (total runtime 15 min.). Methyl 2-acetamidoacrylate (8) elutes at tR of about 4.8 min., sulfonlated product (10) elutes at tR of about 7.8 min., and N-acetylhomophenylalanine methylester (9) elutes at tR of about 9.9 min.
S1.2 – [Ir(dF(CF₃)ppy)₂(dNMe₃bpy)]Cl₃ (2)

3 (59 mg, 1 eq., 0.039 mmol) was dissolved in 40 mL 2-ethoxyethanol and 5 mL water (heat, if necessary, for complete dissolution). 4 (36 mg, 2 eq., 0.079 mmol) was predissolved in 5 mL water and added dropwise. The resulting mixture was heated at 150 °C overnight. The solvent was removed by rotary evaporation. The residue was taken up in methanol and filtered over cotton to remove insoluble solids. The bulk methanol was removed by concentration under reduced pressure and the remainder 2-ethoxyethanol was removed by lyophilisation. The resulting crude yellow solid was recrystallized by vapour diffusion of ether to methanol in the fridge to give 2 (30 mg, 30%) as yellow solid. Ion-exchange chromatography provided 2 as the pure chloride salt. ¹H-NMR (600 MHz, D₂O): δ 3.27 (s, 18H), 4.82 (s, 4H), 5.93 (s, 2H), 7.74 (s, 2H), 7.85 (s, 2H), 8.27 (s, 2H), 8.36 (s, 2H), 8.58 (s, 2H), 8.98 (s, 2H) ppm; ¹³C-NMR (150 MHz, D₂O): δ 53.3, 66.9, 99.8, 114.2, 121.0, 122.8, 124.0, 125.4, 126.8, 129.1, 132.4, 137.2, 140.2, 145.8, 152.4, 156.1, 161.5, 163.5, 167.2 ppm; ¹⁹F-NMR (376 MHz, D₂O): δ -107.0, -104.1, -62.9 ppm; MS (ESI pos, HCOOH) m/z 336.4208 ([M⁺]3+, calc: 336.4204).

S1.3 – [(dF(CF₃)ppy)₂-Ir-μ-Cl]₂ (3)

Prepared as described by Molander et al. ¹: 11 (501 mg, 2.2 eq., 1.93 mmol) and iridium(III)chloride hydrate (261 mg, 1 eq., 0.87 mmol) were suspended in 14 mL 2-ethoxyethanol and 4.7 mL water. The mixture was heated at 120 °C overnight. After cooling to room temperature, the mixture was diluted with 20 mL water. The yellow precipitate was collected by filtration, washed with water and diethyl ether to give 3 (457 mg, 70%) as yellow solid. ¹H-NMR (400 MHz, CDCl₃): δ 5.07 (m, 4H), 6.43 (m, 4H), 8.05 (m, 4H), 8.46 (m, 4H), 9.51 (m, 4H) ppm.

S1.4 – 4,4′-bis(trimethylammoniummethyl)-2,2′-bipyridine (4)

Prepared as described by Ji et al. ²: 7 (230 mg, 1 eq., 0.67 mmol) was suspended in 8 mL ethanol. Aqueous trimethylamine (33%, 1.3 mL, 3.4 eq., 5.3 mmol) was added dropwise. The solution was stirred until it became clear (30 min.) and cloudy again (30 min.). Evaporation of the solvent and washing with ethanol gave 4 (315 mg, quantitative) as white solid. ¹H-NMR (400 MHz, D₂O): δ 3.24 (s, 18H), 4.71 (s, 4H), 7.77 (d, J = 4.9 Hz, 2H), 8.36 (s, 2H), 8.87 (d, J = 4.9 Hz, 2H) ppm; ¹³C-NMR (101 MHz, D₂O): δ 55.7, 70.4, 128.4, 130.9, 140.7, 152.9, 158.2 ppm; HRMS (ESI pos, HCOOH) m/z 150.115 ([M⁺]₂⁺, calc: 150.115); Elemental analysis calcd (%) for C₁₈H₂₈N₄Br₂ · 3 H₂O: C, 42.0; H, 6.7; N, 10.9; found: C, 41.7; H, 6.2; N, 10.65.

S1.5 – 4,4′-bis(hydroxymethyl)-2,2′-bipyridine (6)

Prepared as described by Trabolsi et al. ³: 4,4′-bis(methoxycarbonyl)-2,2′-bipyridine (890 mg, 1 eq., 3.25 mmol) was suspended in 5 mL absolute ethanol. Sodium borohydride (2.4 g, 20 eq., 65 mmol) was added as one portion. After refluxing for 3 hours, the mixture was cooled to room temperature and carefully diluted with saturated NH₄Cl (aq.). Ethanol was removed in vacuo and the aqueous layer was extracted with ethyl acetate (note: product does not dissolve in dichloromethane!). Drying over Na₂SO₄ and removal of the solvent gave 6 (635 mg, 90%) as a white solid. ¹H-NMR (400 MHz, (CD₃)₂SO): δ 4.63 (d, J = 5.5 Hz, 4H), 5.50 (t, J = 5.5 Hz, 2H), 7.37 (d, J = 4.8 Hz, 2H), 8.39 (s, 2H), 8.60 (d, J = 4.8 Hz, 2H) ppm; ¹³C-NMR (101 MHz, (CD₃)₂SO): δ 61.7, 117.7, 121.4, 148.9, 152.8, 155.2 ppm; HRMS (ESI pos, HCOOH) m/z 217.097 ([M+H]+, calc: 217.097), 239.079 ([M+Na]+, calc: 239.079); Elemental analysis calcd (%) for C₁₂H₁₂N₂O₂ · HCl: C, 57.0; H, 5.2; N, 11.1; found: C, 56.6; H, 5.15; N, 11.0.
S1.6 – 4,4′-bis(bromomethyl)-2,2′-bipyridine (7)

Prepared as described by Trabolsi et al. 3: 6 (489 mg, 1 eq., 2.3 mmol) was dissolved in 12 mL HBr (48% in H2O, 47 eq., 107 mmol). After refluxing overnight, the mixture was cooled to room temperature and diluted with 20 mL water. The mixture was basified to pH 10 with 10 M NaOH (aq.). The formed precipitate was filtered off, washed with water, and dissolved in chloroform. Drying over Na2SO4, removal of the solvent under reduced pressure, and purification by column chromatography (SiO2, dichloromethane / ethyl acetate, 4:1, Rf = 0.67) gave 7 (481 mg, 61%) as white solid. 1H-NMR (400 MHz, CDCl3): δ 2.12 (s, 3H), 3.84 (s, 3H), 5.87 (s, 1H), 1.96 (s, 3H), 2.11-2.20 (m, 1H), 2.57-2.68 (m, 2H), 3.67 (s, 3H), 4.63 (q, J = 7.7 Hz, 1H), 7.37 (dd, J = 5.0, 1.7 Hz, 2H), 8.44 (s, 2H), 8.67 (d, J = 5.0 Hz, 2H) ppm; 13C-NMR (101 MHz, CDCl3): δ 23.0, 31.7, 33.8, 52.0, 52.3, 126.2, 128.5, 140.7, 141.8 ppm; HRMS (ESI pos, HCOOH) m/z 300.090 ([M+H]+, calc: 300.092); Elemental analysis calc (%) for C12H10Br2N2: C, 42.1; H, 2.95; N, 8.2; found: C, 41.9; H, 2.9; N, 8.4.

S1.7 – methyl 2-acetamidoacrylate (8)

Prepared as described by Rault et al. 4: To a 100 mL round-bottom flask, equipped with a Dean-Stark trap, was added consecutively acetamide (1 g, 1 eq., 16.9 mmol), methyl pyruvate (1.3 mL, 0.9 eq., 15.2 mmol), and 30 mL toluene. Catalytic amounts of p-toluenesulfonic acid (3.3 mg, 0.001 eq., 0.018 mmol) were added. Heating under reflux for 40 hours turned the colourless solution into a yellow reaction mixture, which was concentrated in vacuo. The obtained crude product was redissolved in dichloromethane and washed with saturated sodium bicarbonate (aq.) and water. The organic layer was dried over anhydrous MgSO4, filtered, and concentrated under reduced pressure. Purification by column chromatography (SiO2, dichloromethane / ethyl acetate, 4:1, Rf = 0.71 in ethyl acetate) gave 8 (764 mg, 35%) as a white crystalline solid. 1H-NMR (400 MHz, CDCl3): δ 2.12 (s, 3H), 3.84 (s, 3H), 5.87 (s, 1H), 6.59 (s, 1H), 7.72 (br s, 1H) ppm; 13C-NMR (101 MHz, CDCl3): δ 24.8, 53.1, 108.8, 131.0, 164.8, 168.9 ppm; HRMS (ESI pos, HCOOH) m/z 144.065 ([M+H]+, calc: 144.066); Elemental analysis calc (%) for C6H9NO3: C, 50.3; H, 6.3; N, 9.8; found: C, 50.2; H, 6.3; N, 9.7.

S1.8 – N-acetylhomophenylalanine methylester (9)

Prepared as described by Jeminet et al. 5: To a white suspension of L-homophenylalanine (500 mg, 1 eq., 2.79 mmol) in 2.8 mL anhydrous methanol was added thionyl chloride (0.33 mL, 1.6 eq., 4.46 mmol) at -10 °C. The clear solution was stirred for 1 hour at -10 °C and for 16 hours at room temperature. The solvent was removed in vacuo and the white solid aminoester hydrochloride was suspended in 18.6 mL dry dichloromethane. To the white suspension was added triethylamine (0.86 mL, 2.2 eq., 6.14 mmol) at room temperature and acetyl chloride (0.22 mL, 1.1 eq., 3.07 mmol) at 0 °C. The off-white suspension was stirred for 2 hours at room temperature and filtered to remove the triethylamine hydrochloride precipitate. The filtrate was concentrated under reduced pressure. The crude product was purified by column chromatography (SiO2, dichloromethane / ethyl acetate, 25→50%, Rf = 0.55, KMnO4-stain) to yield 9 (444 mg, 68%) as a yellow sticky solid. 1H-NMR (400 MHz, CDCl3): δ 1.91-2.01 (m, 1H), 1.96 (s, 3H), 2.11-2.20 (m, 1H), 2.57-2.68 (m, 2H), 3.67 (s, 3H), 4.63 (q, J = 7.7 Hz, 1H), 6.42 (br s, 1H), 7.12-7.17 (m, 3H), 7.20-7.30 (m, 2H) ppm; 13C-NMR (101 MHz, CDCl3): δ 23.6, 31.7, 33.8, 52.0, 52.3, 126.2, 128.5, 140.7, 170.1, 173.0 ppm; HRMS (ESI pos, HCOOH) m/z 236.128 ([(M+H)+], calc: 236.128).

S1.9 – methyl acetyl(benzylsulfonyl)alaninate (10)

In a Schlenk flask was suspended 8 (28.6 mg, 1 eq., 0.2 mmol) and zinc benzenesulfinate (225.5 mg, 3 eq., 0.6 mmol) in a solvent mixture of 1 mL ethanol and 1 mL water. The reaction mixture was degassed for 30 min. by bubbling Argon-gas and stirred for 40 hours at room temperature. The white suspension was diluted with 10 mL water and extracted with 3 x 20 mL ethyl acetate. The combined organic layers were washed with 40 mL brine and dried over anhydrous Na2SO4. Removal of the solvent in vacuo gave 10 (12 mg) as a white solid mixed with 8. 1H-NMR (400 MHz, CDCl3): δ 2.03 (s, 3H), 3.46-3.62 (m, 2H), 3.76 (s, 3H), 4.22-4.30 (m, 2H), 4.93 (dt, J = 7.8, 4.8 Hz, 1H), 6.69 (d, J = 7.8 Hz, 1H), 7.40 (s, 5H) ppm; 13C-NMR (101 MHz, CDCl3): δ 23.1, 48.4, 51.9, 53.4, 61.9, 127.3, 129.3, 129.4, 169.7, 170.4 ppm (1 signal missing due to overlap); HRMS (ESI pos, HCOOH) m/z 322.072 ([M+Na]+, calc: 322.072).
S1.10 – 2-(2,4-difluorophenyl)-5-(trifluoromethyl)pyridine (11)
Prepared as described by Weaver et al. 6: 2-chloro-5-(trifluoromethyl)pyridine (317 mg, 1 eq., 1.75 mmol), 2,4-difluorophenylboronic acid (331 mg, 1.2 eq., 2.1 mmol), triphenylphosphine (45 mg, 0.1 eq., 0.175 mmol), and potassium carbonate (649 mg, 2.7 eq., 4.7 mmol) were dissolved in 2 mL 1,2-dimethoxyethane. After degassing by N2 bubbling for 15 min., palladium(II)acetate (10 mg, 0.025 eq., 0.043 mmol) was added and the mixture was degassed by N2 bubbling for another 15 min. After heating under reflux overnight, the mixture was cooled to room temperature and diluted with dichloromethane. The organic layer was washed with water and brine. Drying over Na2SO4, removal of the solvent *in vacuo*, and purification by column chromatography (SiO2, heptane / ethyl acetate, 0% → 3%, Rf = 0.57) gave 11 (322 mg, 71%) as white solid. 1H-NMR (400 MHz, CDCl3): δ 6.95 (m, 1H), 7.05 (m, 1H), 7.92 (m, 1H), 8.01 (m, 1H), 8.10 (m, 1H), 8.97 (s, 1H) ppm; 13C-NMR (101 MHz, CDCl3): δ 104.8, 112.4, 123.8, 132.6, 133.9, 146.7, 155.9, 159.8, 162.4, 162.8, 165.4 ppm; HRMS (ESI pos, HCOOH) m/z 260.050 ([M+H]+, calc: 260.049); Elemental analysis calcd (%) for C12H6F5N: C, 55.6; H, 2.3; N, 5.4; found: C, 55.6; H, 2.3; N, 5.2.

S1.11 – 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (12)
Prepared as described by Marfey 7: L-alaninamide hydrochloride (125 mg, 1.17 eq., 1.00 mmol) was dissolved in 1.03 mL 1 M sodium hydroxide (aq.). Immediately, 15.8 mL acetone and 3 g anhydrous MgSO4 were added. The white suspension was stirred at room temperature for 4 hours. Subsequently, the reaction mixture was filtered to remove MgSO4, which was washed twice with acetone. 1,5-Difluoro-2,4-dinitrobenzene (176 mg, 1 eq., 0.86 mmol) was dissolved in 4 mL acetone. To this yellow solution was added dropwise the acetone solution of L-alaninamide hydrochloride under magnetic stirring. The reaction mixture was stirred for an additional 30 min. and subsequently 25 mL water was added. Part of the solvent was removed under reduced pressure until a yellow suspension was formed. The precipitate was filtered, washed first with water/acetone (2:1), and then with water. The product was dried under vacuum in the dark to yield 12 (234 mg, quantitative) as a yellow solid. 1H-NMR (400 MHz, (CD3)2SO): δ 1.45 (d, J = 6.8 Hz, 3H), 4.39 (p, J = 6.8 Hz, 1H), 6.95 (d, J = 14.4 Hz, 1H), 7.50 (br s, 1H), 7.73 (br s, 1H), 8.90 (d, J = 8.1 Hz, 1H), 9.10 (d, J = 6.4 Hz, 1H) ppm; 13C-NMR (101 MHz, (CD3)2SO): δ 18.3, 51.6, 102.1, 125.1, 127.4, 147.6, 157.7, 160.4, 172.3 ppm; HRMS (ESI pos, HCOOH) m/z 273.063 ([M+H]+, calc: 273.063); Elemental analysis calcd (%) for C9H9FN4O5: C, 39.7; H, 3.3; N, 20.6; found: C, 39.8; H, 3.1; N, 20.5.

S1.12 – Reaction scheme synthesis of glucose decorated iridium(III) photoredox catalyst 15

![Scheme S1: Synthesis of glucose decorated iridium(III) photoredox catalyst 15.](image)
S1.13 – 4,4′-bis[(2,3,4,6-tetra-O-acetyl-β-D-glycopyranosyl)thiomethyl]-2,2′-bipyridine (13)

Prepared as described by Yang et al. 8: 1-thio-β-D-glucosetetraacetate (500 mg, 2.1 eq., 1.37 mmol), sodium carbonate (473 mg, 7 eq., 4.47 mmol), and 6 (218 mg, 1 eq., 0.64 mmol) were dissolved in 5 mL DMF. The mixture was stirred for 48 hours at room temperature. After removal of the solvent in vacuo, water was added, and the mixture was extracted to ethyl acetate. Drying over Na2SO4, removal of the solvent, and purification by column chromatography (SiO2, ethyl acetate / heptane, 3:1, Rf = 0.5) gave 13 (557 mg, 95%) as white solid. 1H-NMR (400 MHz, CDCl3): δ 1.97 (s, 6H), 2.00 (s, 6H), 2.02 (s, 6H), 2.08 (s, 6H), 3.66-3.70 (m, 2H), 3.84 (d, J = 13.5 Hz, 2H), 4.03 (d, J = 13.5 Hz, 2H), 4.16-4.26 (m, 4H), 4.37 (d, J = 9.8 Hz, 2H), 4.98-5.25 (m, 6H), 7.30 (dd, J = 5.0, 1.5 Hz, 2H), 8.39 (s, 2H), 8.60 (d, J = 5.0 Hz, 2H) ppm; 13C-NMR (100 MHz, CDCl3): δ 20.7, 20.8, 20.9, 21.2, 32.9, 62.1, 68.3, 69.9, 73.8, 76.1, 82.1, 121.8, 124.4, 147.8, 149.4, 155.8, 169.5, 169.6, 170.2, 170.7 ppm; HRMS (ESI pos, HCOOH) m/z 909.244 ([M+H]+, calc: 909.215).

S1.14 – 4,4′-bis[(β-D-glycopyranosyl)thiomethyl]-2,2′-bipyridine (14)

Prepared as described by Yang et al. 8: 13 (230 mg, 1 eq., 0.25 mmol) was dissolved in 4.5 mL methanol. Sodium methoxide (20 mg, 1.36 eq., 0.34 mmol) was added and the mixture was stirred for 16 hours at room temperature. The precipitate was collected by filtration and washed with cold methanol to give 14 (115 mg, 80%) as a white solid. 1H-NMR (400 MHz, (CD3)2SO): δ 3.07 (s, 8H), 3.43-3.52 (m, 2H), 3.69-3.80 (m, 2H), 3.89-3.93 (m, 2H), 4.01-4.10 (m, 4H), 4.67 (t, J = 5.0 Hz, 2H), 4.92 (s, 2H), 5.01 (s, 2H), 5.10-5.19 (m, 2H), 7.35-7.54 (m, 2H), 8.40 (s, 2H), 8.60 (d, J = 5.0 Hz, 2H) ppm; 13C-NMR (101 MHz, (CD3)2SO): δ 31.4, 61.3, 70.1, 73.2, 78.2, 81.1, 83.1, 121.0, 124.6, 148.9, 149.2, 155.2 ppm; HRMS (ESI pos, HCOOH) m/z 573.157 ([M+H]+, calc: 573.157).

S1.15 – [Ir(dF(CF3)ppy)2(dGlubpy2)] (15)

3 (97 mg, 1 eq., 0.065 mmol) and 14 (75 mg, 2 eq., 0.131 mmol) were dissolved in 30 mL dichloromethane / methanol (1:1 v/v). The mixture was refluxed for 16 hours. After removal of the solvent in vacuo, the crude was taken up in 5 mL ethanol. The formed white precipitate was filtered off and the filtrate was concentrated under reduced pressure. Repeating the last step twice gave 15 (110 mg, 64%) as yellow solid. 1H-NMR (400 MHz, CD3OD): δ 3.16-3.30 (m, 8H), 3.50-3.59 (m, 2H), 3.80-3.86 (m, 2H), 4.02-4.06 (m, 2H), 4.17-4.23 (m, 2H), 4.28-4.33 (m, 2H), 5.74-5.77 (m, 2H), 6.77-6.83 (m, 2H), 7.12-7.16 (m, 4H), 7.26-7.36 (m, 4H) ppm; 13C-NMR (101 MHz, CD3OD): δ 33.1, 63.1, 71.7, 74.4, 79.5, 82.3, 85.2, 100.5, 115.2, 125.0, 125.2, 127.9, 130.6, 138.9, 147.1, 149.8, 151.8, 156.0, 157.1, 162.7, 165.0, 169.2 ppm; HRMS (ESI pos, HCOOH) m/z 1281.183 ([M]+, calc: 1281.181).

S1.16 – zinc 4-fluorobenzylsulfinate (16)

Zinc powder (1.10 g, 7 eq., 16.8 mmol) was suspended in 2.7 mL water. The dark grey suspension was cooled in an ice-water bath and (4-fluorophenyl)-methanesulfonyl chloride (500 mg, 1 eq., 2.4 mmol) was added in portions. The reaction mixture was allowed to slowly warm to room temperature over 3 hours. Subsequently, 5 mL water was added and the suspension was stirred for another 30 min. Filtration of the reaction mixture over a glass filter with ~0.5 cm sand, washing of the residue with 20 mL water, and removal of the solvent in vacuo yielded 16 (401 mg, ~70%) as a mixture with sulfonate (sulfinate:sulphone, 88:12) as white solid. 1H-NMR (400 MHz, D2O): δ 3.62 (s, 4H), 7.12-7.16 (m, 4H), 7.26-7.36 (m, 4H) ppm; 13C-NMR (101 MHz, D2O): δ 69.3, 117.9, 130.5, 134.2, 164.6 ppm; 19F-NMR (376 MHz, D2O): δ -116.4 ppm.
S1.17 – zinc 4-trifluoromethylbenzylsulfinate (17)

Zinc powder (354 mg, 7 eq., 5.4 mmol) was suspended in 0.9 mL methanol. The dark grey suspension was cooled in an ice-water bath and 4-trifluoromethylbenzyl sulfonyl chloride (200 mg, 1 eq., 0.77 mmol) was added in portions. The reaction mixture was allowed to slowly warm to room temperature over 3 hours. Subsequently, 2 mL methanol was added and the suspension was stirred for another 30 min. Filtration of the reaction mixture over a glass filter with ~0.5 cm sand, washing of the residue with 25 mL methanol, and removal of the solvent under reduced pressure yielded 17 (239 mg) as a mixture with sulfonate (sulfinate:sulphone, 79:3 1) as white solid. 1H-NMR (400 MHz, D2O): δ 3.72 (s, 4H), 7.45 (d, J = 8.1 Hz, 4H), 7.71 (d, J = 8.1 Hz, 4H) ppm; 13C-NMR (101 MHz, D2O): δ 70.0, 127.9, 128.0, 131.3, 132.9, 138.9 ppm; 19F-NMR (376 MHz, D2O) δ -62.2 ppm.

S1.18 – zinc tolylsulfinate (18)

Zinc powder (1.118 g, 7 eq., 17.1 mmol) was suspended in 2.7 mL water. The dark grey suspension was cooled in an ice-water bath and 4-methylbenzylsulfonyl chloride (500 mg, 1 eq., 2.44 mmol) was added in portions. The reaction mixture was allowed to slowly warm to room temperature over 3 hours. Subsequently, 5 mL water was added and the suspension was stirred for another 30 min. Filtration of the reaction mixture over a glass filter with ~0.5 cm sand, washing of the residue with 20 mL water, and removal of the solvent in vacuo yielded 18 (42 mg) as a mixture with sulfonate (sulfinate:sulphone, 84:16) as white solid. 1H-NMR (400 MHz, D2O): δ 2.34 (s, 6H), 3.61 (s, 4H), 7.24 (q, J = 7.9 Hz, 8H) ppm; 13C-NMR (101 MHz, D2O): δ 22.7, 70.1, 131.8, 132.5, 132.9, 140.0 ppm.

S1.19 – Stock-solution preparation for photoredox catalysis on Dha-monomer

| compound                  | molarity (mM) | solvent                  |
|---------------------------|---------------|--------------------------|
| methyl 2-acetamidoacrylate (8) | 10            | 50 mM Na2HPO4 buffer (pH 7) or 0.1% AcOH in H2O |
| zinc benzyllsulfinate      | 10            | 50 mM Na2HPO4 buffer (pH 7) or 0.1% AcOH in H2O |
| riboflavin                 | 1             | H2O                      |
| [Ir(dF(CF3)ppy)2(dNMe3bpy)]Cl3 (2) | 1             | H2O                      |

*Table S1: Stock-solutions for photoredox catalysis on Dha-monomer (8).*

S1.20 – General procedure of photocatalysis on protected Dha 8

Catalysis was performed in phosphate buffer (50 mM Na2HPO4, pH = 7) or 0.1% acetic acid in H2O with a final concentration of 1 mM methyl 2-acetamidoacrylate (8), 3 mM zinc benzyllsulfinate, and 20 µM photoredox catalyst. A typical reaction was set up as follows: To a 10 mL Schlenk flask was added consecutively 300 µL zinc benzyllsulfinate stock-solution, 20 µL photoredox catalyst stock-solution, and 100 µL methyl 2-acetamidoacrylate (8) stock-solution. The final volume of the reaction mixture was adjusted to 1 mL by adding aqueous medium. The reaction mixture was subjected to a five-cycle freeze-pump-thaw procedure under N2-atmosphere. Subsequently, the reaction mixture was irradiated with blue LEDs (407.5 nm, approximately 7 cm distance) for either 3 or 16 hours. The reaction mixture was extracted with 3 x 5 mL ethyl acetate, dried over Na2SO4, filtered, and concentrated in vacuo. The crude was dissolved in 0.5 mL 1,4-dioxane and analysed by UPLC/MS TQD using the method as described in S1.1.
Figure S1: Extracted ion chromatograms (EIC) of the pure synthesised reference compounds combined in one graph. In blue, methyl 2-acetamidoacrylate (8) is illustrated with $[M+H]^+ = 144$. In red, homophenylalanine (9) is depicted with $[M+H]^+ = 236$. In black, sulfonylated product (10) is depicted with $[M+H]^+ = 300$.

Figure S2: Extracted ion chromatograms (EIC) of the reaction mixtures obtained by reacting 8 with zinc benzylsulfinate in the presence of the water-soluble iridium photoredox catalyst 2. In blue, 8 is illustrated with $[M+H]^+ = 144$. In red, 9 is depicted with $[M+H]^+ = 236$. In black, 10 is depicted with $[M+H]^+ = 300$. A = 3 hours irradiation, 50 mM phosphate buffer and B = 3 hours irradiation, 0.1% AcOH in H$_2$O.

Figure S3: Extracted ion chromatograms (EIC) of the reaction mixtures obtained by reacting 8 with zinc benzylsulfinate in the presence of riboflavin. In blue, 8 is illustrated with $[M+H]^+ = 144$. In red, 9 is depicted with $[M+H]^+ = 236$. In black, 10 is depicted with $[M+H]^+ = 300$. A = 3 hours irradiation, 50 mM phosphate buffer and B = 3 hours irradiation, 0.1% AcOH in H$_2$O.
S1.21 – Control experiments Dha derivative 8

To prove that the presence of photoredox catalyst in combination with irradiation with blue LED (407.5 nm), ultimately formed homophenylalanine (9) from the protected Dha (8), several control experiments were conducted. Reactions were performed with photoredox catalyst without irradiation, without any photoredox catalyst with irradiation, with photoredox catalyst with irradiation, and with the addition of TEMPO in both types of aqueous media.

Scheme S3: General reaction scheme of control experiments of photoredox catalysed modification of 8.

Reactions performed in 0.1% AcOH in H₂O

Figure S4: Extracted ion chromatograms (EIC) of the reaction mixtures obtained by reacting 8 with zinc benzylsulfinate in the presence of photoredox catalyst without irradiation with blue LED (407.5 nm). In blue, 8 is illustrated with [M+H]+ = 144. In red, 9 is depicted with [M+H]+ = 236. In black, 10 is depicted with [M+H]+ = 300. A = water-soluble iridium photoredox catalyst 2, B = riboflavin.

Figure S5: EIC of the reaction mixtures obtained by reacting 8 with zinc benzylsulfinate without any photoredox catalyst and with irradiation with blue LED (407.5 nm) for 16 hours. In blue, 8 is illustrated with [M+H]+ = 144. In red, 9 is depicted with [M+H]+ = 236. In black, 10 is depicted with [M+H]+ = 300.
Figure S6: EICs of the reaction mixtures obtained by reacting 8 with zinc benzylsulfinate in the presence of photoredox catalyst and TEMPO upon irradiation with blue LED (407.5 nm). In blue, 8 is illustrated with [M+H]^+ = 144. In red, 9 is depicted with [M+H]^+ = 236. In black, 10 is depicted with [M+H]^+ = 300. A = water soluble iridium photoredox catalyst 2, B = riboflavin.

Reactions performed in phosphate buffer (50 mM Na2HPO₄, pH = 7)

Figure S7: EICs of the reaction mixtures obtained by reacting 8 with zinc benzylsulfinate in the presence of photoredox catalyst without irradiation with blue LED (407.5 nm). In blue, 8 is illustrated with [M+H]^+ = 144. In red, 9 is depicted with [M+H]^+ = 236. In black, 10 is depicted with [M+H]^+ = 300. A = water-soluble iridium photoredox catalyst 2, B = riboflavin.

Figure S8: EICs of the reaction mixture obtained by reacting 8 with zinc benzylsulfinate without any photoredox catalyst and with irradiation with blue LED (407.5 nm). In blue, 8 is illustrated with [M+H]^+ = 144. In red, 9 is depicted with [M+H]^+ = 236. In black, 10 is depicted with [M+H]^+ = 300. A = irradiation for 16 hours, B = irradiation for 72 hours.

Figure S9: EICs of the reaction mixtures obtained by reacting 8 with zinc benzylsulfinate in the presence of photoredox catalyst and TEMPO upon irradiation with blue LED (407.5 nm). In blue, 8 is illustrated with [M+H]^+ = 144. In red, 9 is depicted with [M+H]^+ = 236. In black, 10 is depicted with [M+H]^+ = 300. A = water-soluble iridium photoredox catalyst 2, B = riboflavin.
S1.22 – Larger scale photocatalysis on Dha derivative 8

Scheme S4: Reaction scheme of photoredox catalysed modification of methyl 2-acetamidoacrylate (8).

Catalysis was performed in 0.1% acetic acid in H2O with a final concentration of 10 mM methyl 2-acetamidoacrylate (8), 30 mM zinc benzylsulfinate, and 0.2 mM [Ir(dF(CF3)ppy)2(dNMe3bpy)]Cl3 (2). The reaction was set up as follows: In a 100 mL three-necked flask was dissolved 50 mg (0.35 mmol, 1 eq.) methyl 2-acetamidoacrylate (8) and 7.8 mg (0.007 mmol, 0.02 eq.) [Ir(dF(CF3)ppy)2(dNMe3bpy)]Cl3 (2) in 35 mL 0.1% AcOH in H2O. The yellow solution was degassed by bubbling Ar-gas for 1 hr. After 50 min. of degassing, blue LEDs (407.5 nm, approximately 5 cm distance) were switched on and the system was cooled with pressurized airflow. After 60 min. degassing, 394 mg (1.05 mmol, 3 eq.) zinc benzylsulfinate was added in portions of 50-60 mg every 15 minutes, while irradiating with blue LEDs degassing by bubbling Ar-gas, and cooling with pressurized airflow. The reaction mixture was irradiated with blue LEDs for 3.5 hours. The yellow suspension was extracted with 3 x 50 mL ethyl acetate, dried over MgSO4, filtered, and concentrated in vacuo. The crude product was purified by column chromatography (SiO2, dichloromethane / ethyl acetate, 3:2, Rf = 0.34, KMnO4-stain) to yield 9 (22.4 mg, 27%) as a yellow sticky solid. 1H-NMR (400 MHz, CDCl3): δ 1.95-2.03 (m, 1H), 1.97 (s, 3H), 2.08-2.26 (m, 1H), 2.52-2.72 (m, 2H), 3.69 (s, 3H), 4.66 (q, J = 7.3 Hz, 1H), 6.02 (s, 1H), 7.14-7.19 (m, 3H), 7.21-7.34 (m, 2H) ppm. This NMR is in agreement with the NMR in S1.8 for the synthesis of 9 (except for the NH peak, which is shifted).
S2 Molecular Biology

S2.1 – General remarks

E. coli strains XL1 Blue and BL21 C43(DE3) (Stratagene) were used for routine cloning and protein production, respectively. Streptag®-Strep-Tactin® purification columns were purchased from IBA. Ni sepharose™ purification column material was purchased from GE Healthcare. PCR reactions were carried out using an Eppendorf Mastercycler Personal apparatus. DNA sequencing was carried out by GATC-Biotech and Eurofins Genomics. Primers were synthesised by Biotez and Eurofins Genomics. Restriction endonucleases were purchased from New England Biolabs. T4 DNA ligase, DNA gel Extraction Kit, and Plasmid Purifying kit were purchased from Roche. Pfu Turbo polymerase was purchased from Invitrogen. Reaction mixtures, peptides, and proteins were analysed by liquid-chromatography mass-spectrometry (LC-MS) performed on a Waters Acquity Ultra Performance LC with TQD mass detector (ESI+). UPLC grade 0.1% formic acid (FA) in H2O (solvent A) and 0.1% FA in acetonitrile (solvent B) were used as eluents. Separation of nisin photoredox reactions was achieved with a reversed-phase UPLC column (Acquity UPLC BEH C8, 1.7 µm, 2.1 mm x 150 mm) at 40 °C. There was made use of a linear gradient: 90% A to 50% A over 8 min., then to 5% A over 1 min. (total runtime 15 min.). Nisin elutes at tR of about 6.5-8.5 min. Separation of Marfey’s adducts was achieved with a reversed-phase UPLC column (Acquity UPLC HSS T3, 1.8 µm, 2.1 mm x 150 mm) at 40 °C. There was made use of a linear gradient: 80% A to 40% A over 15 min., then to 5% A over 1 min. (total runtime 20 min.). Marfey derivatised dehydroalanine adducts elute at tR of about 13.7 and 15.6 min. Separation of proteins (SUMO and sfGFP) was achieved with a reversed-phase UPLC column (Acquity UPLC Protein BEH C4, 1.7 µm, 300 Å, 2.1 mm x 150 mm) at 40 °C. There was made use of a linear gradient: 90% A to 50% A over 8 min., then to 5% A over 1 min. (total runtime 15 min.). SUMO elutes at tR of about 7.2-8 min. and sfGFP elutes at tR of about 7.4-8.5 min. Obtained charge density spectra were deconvoluted with the algorithm MagTran. Optical density of the bacterial cultures was measured with an Amersham Biosciences Ultrospec 10. Protein concentrations were measured on a Thermoscientific Nanodrop 2000. Denaturing polyacrylamide gel for SDS/PAGE was casted from stock solution of 30% (w/v) 29:1 monomer:crosslinker acrylamide/N,N-methyl-bisacrylamide in Tris-Cl/SDS buffer (= 3M Tris-base, 10 mM SDS, pH 8.45) and 15% glycerol (w/v). Polymerisation was started with 10% ammonium persulfate (APS) and N,N,N,N-tetramethylethylenediamine (TEMED). Denaturing gels were run in Tris-Tricine-SDS buffer (cathode: 1 M Tris-base, 1 M Tricine, 3 mM SDS, anode: 200 mM Tris-base, pH 8.9) at 110-150 V for 1-2 hours. Gel staining was done by Coomassie® stain with InstantBlue™. Vivaspin spinfilters were purchased from Sartorius. Graphic representation of SUMO was taken from PDB 1WM2. Graphic representation of sfGFP was taken from PDB 6B3P. Microcav reactions were done in a CEM Discover SP microwave oven. Eppendorf vials were concentrated in Eppendorf® Concentrator Plus.

S2.2 – Molecular Biology of SUMO

S2.2.1 – Construction of plasmid pET17b_SUMO_G98C

The pET17b_SUMO_G98C plasmid was derived from the commercially available plasmids pET17B (Novagen) and the Champion™ pET SUMO Expression System (Invitrogen). The SUMO gene, including an N-terminal Strep-tag® and C-terminal cysteine-alanine addition, was amplified by PCR using the following primers:

**primer 1:** 5’-TAC TAC CAT ATG TGG AGC CAC CCG CAG TTC GAA AAA ATG TCG GAC TCA GAA GTC AAT CAA GAA GCT AAG CCA GAG GTC AAG CCA GAA GTC AAG CCT GAG ACT CAC ATC AAT TTA AAG GTC AAT CAA GAA G-3’ (including Ndel restriction site underlined)

**primer 2:** 5’-GTA GTA GTC CAG GAG TTA CGC GCA ACC AAT CTG TTC TCT GTG AGC CT-3’ (including Xhol restriction site underlined)

PCR cycles were as following: initial denaturation at 94 °C for 5 min. Denaturation at 94 °C for 30 sec., annealing at 55 °C for 30 sec., extension at 72 °C for 90 sec., for 30 cycles. Final extension at 72 °C for 10 min. The obtained PCR product was digested with Ndel and Xhol and inserted between the same sites of the expression vector pET17b.

S2.2.2 – DNA sequence of pET17b_SUMO_G98C construct

5’-CAT ATG TGG AGC CAC CCG CAG TTC GAA AAA ATG TCG GAC TCA GAA GTC AAT CAA GAA GCT AAG CCA GAG GTC AAG CCA GAA GTC AAG CCT GAG ACT CAC ATC AAT TTA AAG GTC TCC GAT GGA TCT TCA GAG ATC TCC TCT AAG ATC AAA AAG ACC ACT CCT TTA AGA AGG CTC ATG AAG GCG TTC GCT AAA AGA CAG GGT AAG AAG ATG GAC TCC TTA AGA TTC TTA TGC TAC GAT GCT AGT AGA ATT CAA GCT GAT CAG ACC CTT GAA GAT TGG GAC ATG GAG GAT AAC GAT ATT ATT GAG GCT CAC AGA GAA CAG ATT GTG TGC GCG TAA CTC GAG-3’ (mutation site red-coloured)
S2.2.3 – Site-directed mutagenesis

Site-directed mutagenesis was performed on the pET17b_SUMO_G98C plasmid and introduced sequentially. Primers used for preparing the mutant are shown in table S2. PCR cycles were as following: initial denaturation at 95 °C for 30 sec., annealing at 55 °C for 1 min., extension at 68 °C for 4.5 min., for 16 cycles. The obtained PCR product was digested with DpnI and used for transformation to *E. coli* BL21 C43(DE3) without further purification.

| primer                     | sequence (5'→3') |
|----------------------------|------------------|
| SUMO_M60C_G98A_forward     | CAG GGT AAG GAA TGC GAC TCC TTA AGA |
| SUMO_M60C_G98A_reverse     | TCT TAA GGA GTC GCA TTC CTT ACC CTG |

Table S2: PCR primers for site-directed mutagenesis of pET17b_SUMO_G98C.

S2.2.4 – Protein expression and purification

Protein expression plasmids of the SUMO constructs were transformed into *E. coli* BL21 C43(DE3) and a single colony was used to inoculate a starter culture of 5 mL fresh LB medium containing 100 µg/mL ampicillin at 37 °C overnight. 2.5 mL of the starter culture was used to inoculate 250 mL of fresh LB medium containing 100 µg/mL ampicillin at 37 °C. When the culture reached the mid-log phase (optical density at 600 nm around 0.6-0.8) isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to a final concentration of 1 mM to induce expression of the target protein. Expressions were done at 37 °C overnight. Cells were harvested by centrifugation (6000 rpm, 20 min., 4 °C, Beckman JLA-10.5), resuspended in phosphate buffer (50 mM NaH2PO4, 150 mM NaCl, 2.5 mM Dl‐dithiotreitol (DTT), pH = 7), and sonicated (75% 200 W) for 10 min. (15 sec. on, 10 sec. off). The disturbed cells were incubated with DNaseI (0.1 mg/mL), phenylmethanesulfonlfyl fluoride (PMSF, 1 mM) and MgCl2 (10 mM) for 30 min. at 30 °C. After centrifugation (12000 rpm, 60 min., 4 °C, Beckman JA-17.5), the supernatant was filtered and equilibrated with 4 mL slurry of Strep-Tactin® column material for 30 min. (mixed on a rotary shaker) at room temperature. The column was washed with 3 x 5 mL of washing buffer (50 mM NaH2PO4, 150 mM NaCl, 2.5 mM DTT, pH = 7) and eluted with 5 x 2 mL of eluting buffer (50 mM NaH2PO4, 150 mM NaCl, 2.5 mM DTT, 2.5 mM D-desthiobiotin, pH = 7). Fractions were analysed on a 12% polyacrylamide Tris-Tricine-SDS gel followed by Coomassie® staining with InstantBlue™. Fractions containing protein were concentrated using a spinfilter (Vivaspin-15). Concentration of protein was determined using the calculated extinction coefficient ε280 = 6,990 M⁻¹ cm⁻¹ (ExPASy Tool 12). Expression yields typically were 20-30 mg/L.

S2.2.5 – Protein sequence

**SUMO_G98C**

M WSHPQFEK MSDSEVNQEA KPEVKPEVKP ETHINLKVS GDSEIFFKIK KTTPLRRLME AFAKRQGKEM DSIRFLYDGI RIQAQTFED LDMDNDIIIE AHREQIQCA (Strep-tag sequence underlined, mutation site red-coloured)

![Figure S10: UPLC/MS TQD spectrum and deconvoluted spectrum of SUMO_G98C.](image-url)
SUMO_M60C_G98A
M WSHPQFEK MSDSEVNQEA KPEVKPEVKP ETHINLKVSD GSSEIFFKIK KTTPLRRLME AFAKRQGKEC
DSILRFYDGI RIQADQTPED LDMDNDDIE AHREQIGAA (Strep-tag sequence underlined, mutation site red-coloured)

Figure S11: UPLC/MS TQD spectrum and deconvoluted spectrum of SUMO_M60C_G98A.

S2.3 – Molecular Biology of sfGFP

S2.3.1 – DNA sequence of sfGFP-pBAD

5'-ATG CGG GGT TCT CAT CAT CAT CAT CAT CAT GGT ATG GCT AGC ATG ACT GGT GGA
CAG CAA ATG GGT CGG GAT CTG TAC GAG AAC CTC TAC TAC TAC GAG CTC ACT GCT GGA
GAC GTA AAC GCC ACC AAG TTC AGC AGC TAC GAG AAC CTG TAC TTC CAG GGC TCG AGC ATG GTG
AGC AAG CTC ACC CTC AAG TAC ATC TGC ACC ACC GGC AAG CTC CCC TGG CCC
ACC CTC GTC ACC ACC CTG ACC AAC GGC CAC AAG TTC AGC GTG CGC GGC GAG GGC GAT GCC ACC AAC
ATG AAG CGC CAC GAC TTC TAC AAG TCC GCC ATG CCC GAA GAC TAC GAG GCG TTC AGC ATG TGC CTG CTG GAG TTC GTG ACC GCC GCC GGG ATC
ACT CAC GCC ATG GAC GAG CTG TAC AAG-3' (mutation site red-coloured)

**Table S3:** PCR primers for site-directed mutagenesis of sfGFP-pBAD.

| primer sequence (5'-3') |
|-------------------------|
| sfGFP_L272C_forward CTC ACG GCA TGG ACG AGT GCT ACA AGT AAG AAT TC |
| sfGFP_L272C_reverse GAA TTC TTA CTT GTA GCA CTC GTC CAT GCC GTC AG |

S2.3.2 – Site-directed mutagenesis

The sfGFP-pBAD plasmid was derived from Addgene (Addgene plasmid #54519; http://n2t.net/addgene:54519; RRID:Addgene_54519). Site-directed mutagenesis was performed on the sfGFP-pBAD plasmid and introduced sequentially. Primers used for preparing the mutant are shown in table S3. PCR cycles were as following: initial denaturation at 95 °C for 5 min., denaturation at 95 °C for 30 sec., annealing at 65 °C for 30 sec., extension at 72 °C for 2 min., for 25 cycles and final extension at 72 °C for 10 min. The obtained PCR product was digested with DpnI and used for transformation to E. coli BL21 C43(DE3) without further purification.

S2.3.3 – Protein expression and purification

The protein expression plasmid of the sfGFP_L272C construct was transformed into E. coli BL21 C43(DE3) and a single colony was used to inoculate a starter culture of 5 mL fresh LB medium containing 100 µg/mL ampicillin at 37 °C overnight. 100 µL of the starter culture was used to inoculate 250 mL of fresh LB medium containing 100 µg/mL ampicillin at 37 °C. When the culture reached OD₆₀₀ = 0.4-0.6, l-arabinose was added to a final concentration of 0.02% to induce expression of the target protein. Expressions were done at 30 °C overnight. Cells were harvested by centrifugation (6000 rpm, 20 min., 4 °C, Beckman JLA-10.5), resuspended in 20 mL phosphate buffer (50 mM NaH₂PO₄, 150 mM NaCl) containing 1 mg/mL egg white lysozyme and a tablet of protease inhibitor cocktail, and incubated for 1 h at 4 °C. The cells were sonicated (75% 200 W) for 10 min. (15 sec. on, 10 sec. off) and the disrupted cells were centrifuged (12000 rpm, 60 min., 4 °C, Beckman JA-17.5).
The supernatant was filtered and equilibrated with 2 mL slurry of Ni Sepharose™ column material for 30 min. (mixed on a rotary shaker) at 4 °C. The column was washed with 2 x 10 mL of washing buffer 1 (50 mM NaH₂PO₄, 150 mM NaCl, pH = 7.5), 2 x 10 mL of washing buffer 2 (50 mM NaH₂PO₄, 150 mM NaCl, 20 mM imidazole, pH = 7.5), and 2 x 10 mL of washing buffer 3 (50 mM NaH₂PO₄, 150 mM NaCl, 40 mM imidazole, pH = 7.5). Thereafter, sfGFP_L272C was eluted with 4 x 5 mL of eluting buffer (50 mM NaH₂PO₄, 150 mM NaCl, 250 mM imidazole, pH = 7.5). Fractions were analysed on a 12% polyacrylamide Tris-Tricine-SDS gel followed by Coomassie® staining with InstantBlue™. Fractions containing protein were concentrated using a spinfilter (Vivaspin-15, cut-off 10,000). Concentration of protein was determined using the calculated extinction coefficient $\varepsilon_{280} = 22,015 \, M^{-1} \, cm^{-1}$ (ExPASy Tool). Expression yields typically were 40-50 mg/L.

S2.3.4 – Protein sequence of sfGFP_L272C

MRGSHHHHHHI GMASMTGQQ Q MGRDLYENLY FQGSSMVSKG EELFTGVVPI LVELDGDVNG
HKFSVRGEGE GDATNGKLTL KFICTTGKLP VPWPTLVTTL TYGVQCFSRY PDHMKRDHF
KSAMPEGYQ ERTISFKDDG TYKTRAEVKF EGDTLVPNIE LGKIDFKEDG NILGKLEYN
FNSHNYITA DKQKNGIKAN FKIRHNVEDG SVQLADHYQQ NTPIGDGPVL LPDNHYLSTQ
SVLSKDPNEK RDHMVLLEFV TAAGITHGMD ECYK (His-tag sequence underlined, mutation site red-coloured)

Figure S12: UPLC/MS TQD spectrum and deconvoluted spectrum of sfGFP_L272C.
S3 Photoredox catalysis on the antimicrobial peptide nisin

S3.1 – Stock-solution preparation for photoredox catalysis on nisin

| compound                                      | molarity (mM) | solvent                  |
|-----------------------------------------------|---------------|--------------------------|
| nisin                                         | 4             | 0.1% AcOH in H₂O         |
| zinc benzylsulfinate                          | 10            | 0.1% AcOH in H₂O         |
| zinc tolylsulfinate (18)*                     | 10            | 0.1% AcOH in H₂O         |
| zinc 4-fluorobenzylsulfinate (16)*            | 10            | 0.1% AcOH in H₂O         |
| zinc 4-trifluoromethylbenzylsulfinate (17)*   | 10            | 0.1% AcOH in H₂O         |
| riboflavin                                    | 0.5           | H₂O                      |
| [Ir(dF(CF₃)ppy)₂(dNMe₃bpy)]Cl₃ (2)            | 1             | H₂O                      |
| TEMPO                                         | 400           | 0.1% AcOH in H₂O / 1,4-dioxane, 1:1 |

* = stock-solutions are made using not purified zinc sulfinate containing corresponding sulphone and ZnCl₂

Table S4: Stock-solutions photoredox catalysis nisin.

S3.2 – General procedure of photoredox catalysis on nisin

Catalysis was performed in 0.1% acetic acid in H₂O with a final concentration of 0.5 mM nisin, 4.5 mM zinc benzylsulfinate, and 25/50/100 µM photoredox catalyst. A typical catalysis reaction was set up as follows: To a screw-cap vial was added 0.1% acetic acid in H₂O, 450 µL zinc benzylsulfinate stock-solution, and photoredox catalyst stock-solution. The solution was degassed by bubbling Ar-gas for 30 min. To the screw-cap vial was added 125 µL nisin. The reaction mixture was degassed another time by flowing Ar-gas for 15 min. The screw-cap vial was sealed, kept under Ar-atmosphere using a balloon, and exposed to blue LED's for 3 or 6 hours at room temperature. After 3 or 6 hours, 37.5 µL TEMPO stock-solution was added and the reaction mixture was stirred for 15 min. at room temperature without irradiation. Subsequently, the reaction mixture was loaded on
a NAP-10 size exclusion chromatography column. The peptide was eluted with 0.1% acetic acid in H₂O and analysed by MALDI-TOF mass spectrometry.

**Figure S13:** MALDI-TOF mass spectrometry spectrum of photoredox catalysed modification of nisin with 5 mol% photoredox catalyst and irradiation for 3 hours. A = water-soluble iridium photoredox catalyst 2, B = riboflavin.

**Figure S14:** MALDI-TOF mass spectrometry spectrum of photoredox catalysed modification of nisin with 10 mol% photoredox catalyst and irradiation for 3 hours. A = water-soluble iridium photoredox catalyst 2, B = riboflavin.

**Figure S15:** MALDI-TOF mass spectrometry spectrum of photoredox catalysed modification of nisin with 20 mol% photoredox catalyst and irradiation for 3 hours. A = water-soluble iridium photoredox catalyst 2, B = riboflavin.
Figure S16: MALDI-TOF mass spectrometry spectrum of photoredox catalysed modification of nisin with 5 mol% photoredox catalyst and irradiation for 6 hours. A = water-soluble iridium photoredox catalyst 2, B = riboflavin.

Figure S17: MALDI-TOF mass spectrometry spectrum of photoredox catalysed modification of nisin with 10 mol% photoredox catalyst and irradiation for 6 hours. A = water-soluble iridium photoredox catalyst 2, B = riboflavin.

S3.3 – Control experiments nisin
To prove that the presence of photoredox catalyst in combination with irradiation with blue LED (407.5 nm), ultimately modifies nisin, several control experiments were conducted. Reactions were performed with photoredox catalyst without irradiation, without any photoredox catalyst with irradiation, and without photoredox catalyst without irradiation. Catalysis was performed following the general procedure described in S3.2 using an irradiation time of 3 hours.

Figure S18: MALDI-TOF mass spectrometry spectrum of photoredox catalysed modification of nisin with 10 mol% photoredox catalyst, but without irradiation. A = water-soluble iridium photoredox catalyst 2, B = riboflavin.
Figure S19: MALDI-TOF mass spectrometry spectrum of photoredox catalysed modification of nisin. A = without photoredox catalyst, but with irradiation, B = without photoredox catalyst and without irradiation.

S3.4 – Relative conversions of photoredox catalysis and control reactions on nisin

Figure S20: Reaction condition screening on nisin with 2 and riboflavin, bar chart represents the amount of (un)modified nisin detected for different catalyst 2 or riboflavin loadings, different irradiation times, and for the control reactions.

| entry | unmodified (%) | singly modified (%) | doubly modified (%) | triply modified (%) | unmodified (%) | singly modified (%) | doubly modified (%) | triply modified (%) |
|-------|----------------|---------------------|--------------------|--------------------|----------------|---------------------|--------------------|--------------------|
| 1     | 10             | 73                  | 17                 | 0                  | 72             | 28                  | 0                  | 0                  |
| 2     | 3              | 82                  | 15                 | 0                  | 43             | 56                  | 1                  | 0                  |
| 3     | 0              | 68                  | 32                 | 0                  | 20             | 78                  | 3                  | 0                  |
| 4     | 1              | 58                  | 39                 | 2                  | 22             | 76                  | 1                  | 0                  |
| 5     | 1              | 44                  | 42                 | 13                 | 14             | 83                  | 3                  | 0                  |
| 6     | 100            | 0                   | 0                  | 0                  | 100            | 0                   | 0                  | 0                  |
| 7     | 100            | 0                   | 0                  | 0                  | 100            | 0                   | 0                  | 0                  |
| 8     | 92             | 8                   | 0                  | 0                  | 92             | 8                   | 0                  | 0                  |

Table S5: Screening of reaction conditions of the water-soluble iridium catalyst 2 and riboflavin photoredox catalysed modification of nisin. Single, double, and triple modifications are observed. The conversion displayed is based on integration of the MALDI-TOF mass spectrometry peaks. Conversion is calculated based on integration of the MALDI-TOF MS peaks of the corresponding product divided by the sum of the areas of all compounds, assuming that ionization is similar for all products, which are structurally very similar.13,14 Water adducts are included within the respective (un)modified nisin peptides.
S3.5 – Marfey analysis of nisin

Scheme S6: General reaction scheme of Marfey analysis on nisin.

The remaining peptide solutions of the photoredox catalysed reactions on nisin were concentrated by lyophilisation, redissolved in 350 µL 6 M HCl (aq.), and transferred to a microwave tube equipped with a stirring bar. The sample was exposed to microwave irradiation for 10 min. at 160 °C with maximum 50 Watt power. The mixture was transferred to an Eppendorf vial and concentrated to dryness using the Eppendorf Concentrator plus. The residue was taken up in 50 µL 1 M NaHCO₃ (aq.) and 10 µL 1% 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (FDAA) in acetone was added. After shaking for 2 hours at 40 °C, the sample was neutralised with 30 µL 2 M HCl (aq.), diluted with 100 µL methanol, and analysed directly by UPLC/MS TQD. Signals obtained at 340 nm absorption were assigned to the corresponding FDAA derivatised amino acids.

Figure S21: Analysis of the site selectivity of the water-soluble iridium 2 photoredox catalysed modification of nisin using Marfey’s method. A = 5 mol%, 3 hours B = 10 mol%, 3 hours, C = 20 mol%, 3 hours.

Figure S22: Analysis of the site selectivity of the riboflavin catalysed modification of nisin using Marfey’s method. A = 5 mol%, 3 hours B = 10 mol%, 3 hours, C = 20 mol%, 3 hours.
S3.6 – NMR studies on nisin

Scheme S7: General reaction scheme of photoredox catalysed modification of nisin.

Catalysis was performed in 0.1% acetic acid in H₂O with a final concentration of 0.5 mM nisin, 4.5 mM zinc benzylsulfinate, and 25/50 µM photoredox catalyst. A typical catalysis reaction was set up as follows: To a Schlenk flask was added 0.1% acetic acid in H₂O, 2.25 mL zinc benzylsulfinate stock-solution, and photoredox catalyst stock-solution. The solution was degassed by bubbling Ar-gas for 30 min. To the Schlenk flask was added 625 µL nisin. The reaction mixture was degassed another time by flowing Ar-gas for 15 min. The Schlenk vial was closed, kept under Ar-atmosphere using a balloon, and exposed to blue LED’s for 6 hours at room temperature. After 6 hours, 187.5 µL TEMPO stock-solution was added and the reaction mixture was stirred for 15 min. at room temperature without irradiation. Subsequently, the reaction mixture was loaded on a NAP-10 size exclusion chromatography column. The peptide was eluted with 0.1% acetic acid in H₂O, the solvent was removed by lyophilisation, and analysed by ¹H-NMR in D₂O using water suppression.
Figure S23: $^1$H-NMR spectrum of nisin, Ir-catalysed modified nisin, and riboflavin modified nisin. Appearance of a multiplet around 7.3 ppm in both Ir- and riboflavin catalysed modified correspond to the protons of the phenyl-ring of the introduced homophenylalanine (HomoPhe) residue.
S3.7 – Reagent scope with various zinc benzylsulfimates on nisin

To prove that the photoredox catalysed modification of nisin also works with different reagents than zinc benzylsulfinate, reactions were performed with various para-substituted zinc benzylsulfimates to incorporate various “unnatural amino acids”. Catalysis was performed following the general procedure described in S3.2, but with an equal amount of a varying zinc benzylsulfinate.

Figure S24: MALDI-TOF mass spectrometry spectrum of photoredox catalysed modification of nisin with 10 mol% photoredox catalyst and irradiation for 3 hours. A = zinc tolylsulfinate (18), B = zinc 4-fluorobenzylsulfinate (16), and C = zinc 4-trifluoromethylbenzylsulfinate (17).
S3.8 – Photoredox catalysis on nisin with excess zinc benzylsulfinate and iridium catalyst 2

In an attempt to increase the amount of triply modified nisin with Dhb-2 adducts, excess of zinc benzylsulfinate and higher iridium catalyst 2 loadings were used in the photoredox catalysed reaction on nisin. Catalysis was performed following the general procedure described in S3.2 using an irradiation time of 6 hours. Use of 10 or 20 mol% iridium catalyst 2 in combination with 36 or 54 equivalents zinc benzylsulfinate resulted in an increased amount of triply modified nisin. Further increase of catalyst or reagent loadings generated a turbid suspension, which decreases the efficiency of photoredox catalysis and results in irreproducible results.

Figure S25: MALDI-TOF mass spectrometry spectrum of photoredox catalysed modification of nisin with photoredox catalyst 2 and irradiation for 6 hours. A = 10 mol% 2 and 36 eq. zinc benzylsulfinate, B = 20 mol% 2 and 36 eq. zinc benzylsulfinate, C = 20 mol% 2 and 54 eq. zinc benzylsulfinate, and D = reaction condition screening on nisin with 2 and excess zinc benzylsulfinate, bar chart represents the amount of (un)modified nisin detected for different catalyst 2 and zinc benzylsulfinate loadings. The conversion represented in the bar chart is based on integration of the MALDI-TOF mass spectrometry peaks. Conversion is calculated based on integration of the MALDI-TOF MS peaks of the corresponding product divided by the sum of the areas of all compounds, assuming that ionization is similar for all products, which are structurally very similar.13,14 Water adducts are included within the respective (un)modified nisin peptides.
S4 Photoredox catalysis on the protein SUMO

S4.1 – Stock-solution preparation for photoredox catalysis on SUMO

| compound                        | molarity (mM) | solvent |
|---------------------------------|---------------|---------|
| zinc benzylsulfinate            | 250           | DMSO    |
| riboflavin                      | 0.5           | H2O     |
| [Ir(dF(CF3)ppy)2(dNMe3bpy)]Cl3 (2) | 1             | H2O     |

*Table S6:* Stock-solutions for photoredox catalysis on SUMO.

S4.2 – General procedure for converting cysteine to dehydroalanine

The cysteine containing protein SUMO_G98C was dissolved in phosphate buffer (50 mM NaH2PO4, 150 mM NaCl, 2.5 mM DTT, pH = 7) and diluted to a concentration of 5 mg/mL. An additional 1-2 mg DTT was added and the protein was incubated for 15 min. The sample was purified by PD MiniTrap™ G-25 size exclusion chromatography. 475 µL of protein solution was combined with 50 µL 2,5-dibromohexanediamide in DMF (20 mg/mL). The mixture was shaken at 750 rpm at 37 °C for 16 hours. Purification by PD MiniTrap™ G-25 size exclusion chromatography and concentration using a spinfilter (Vivaspin-15) gave the dehydroalanine containing protein SUMO_G98Dha in full conversion as was analysed by UPLC/MS TQD.

*Scheme S8:* General reaction scheme of the conversion of SUMO_G98C into SUMO_G98Dha by bis-alkylation-elimination with 2,5-dibromohexanediavmide.

*Figure S26:* UPLC/MS TQD spectrum and deconvoluted spectrum of SUMO_G98Dha.
Conversion of SUMO_M60C into SUMO_M60Dha was achieved following the general procedure described in S4.2, but with an equal amount of SUMO_M60C.

**Scheme S9:** General reaction scheme of the conversion of SUMO_M60C into SUMO_M60Dha by bis-alkylation-elimination with 2,5-dibromohexanediamide.

**Figure S27:** UPLC/MS TQD spectrum and deconvoluted spectrum of SUMO_M60Dha.

**S4.3 – Confirmation of Dha residue by thiol Michael addition**

An aliquot of 3 nmol SUMO_G98Dha was taken and diluted with phosphate buffer (50 mM NaH2PO4, 150 mM NaCl, pH = 7) to 50 µL. 5 µL Mercaptoethanol was added. The mixture was shaken at 750 rpm at 37 °C for 15 min., followed by direct analysis with UPLC/MS TQD.
**Figure S28**: UPLC/MS TQD spectrum and deconvoluted spectrum of thiol Michael addition to SUMO_G98Dha.

Scheme S11: General reaction scheme of thiol Michael addition to dehydroalanine in SUMO_M60Dha.

The Dha residue in SUMO_M60Dha was confirmed by following the general procedure described in S4.3, but with an equal amount of SUMO_M60Dha.

**Figure S29**: UPLC/MS TQD spectrum and deconvoluted spectrum of thiol Michael addition to SUMO_M60Dha.

S4.4 – General procedure of photoredox catalysis on SUMO

Scheme S12: General reaction scheme of iridium photoredox catalysed (2) modification of SUMO_G98Dha.

Catalysis was performed in phosphate buffer (50 mM NaH₂PO₄, 150 mM NaCl, pH = 7) with a final concentration of 100 µM SUMO_G98Dha, 5 mM benzylsulfinate, and 25 or 50 µM iridium photoredox catalyst 2. A typical
catalysis reaction was set up as follows: To a screw-cap vial was added phosphate buffer (50 mM NaH$_2$PO$_4$, 150 mM NaCl, pH = 7), 8 µL zinc benzylsulfinate stock-solution, and 10 or 20 µL iridium photoredox catalyst 2 stock-solution. The solution was degassed by bubbling Ar-gas for 30 min. To the screw-cap vial was added SUMO_G98Dha to obtain a final reaction volume of 400 µL. The reaction mixture was degassed another time by flowing Ar-gas for 15 min. The screw-cap vial was sealed, kept under Ar-atmosphere using a balloon, and exposed to blue LED’s for 1 hour at room temperature. After 1 hour, the reaction mixture was loaded on a PD MiniTrap™ G-25 size exclusion chromatography column. The protein was eluted with phosphate buffer and analysed by UPLC/MS TQD.

**Figure S30:** UPLC/MS TQD spectrum and deconvoluted spectrum of photoredox catalysed modification of SUMO_G98Dha with 50 mol% water-soluble iridium photoredox catalyst 2.

**Figure S31:** UPLC/MS TQD spectrum and deconvoluted spectrum of photoredox catalysed modification of SUMO_G98Dha with 25 mol% water-soluble iridium photoredox catalyst 2.

**Scheme S13:** General reaction scheme of iridium photoredox catalysed (2) modification of SUMO_M60Dha.

Catalysis was performed following the general procedure described in S4.4, but with an equal amount of SUMO_M60Dha.
Figure S32: UPLC/MS TQD spectrum and deconvoluted spectrum of photoredox catalysed modification of SUMO_M60Dha with 50 mol% water-soluble iridium photoredox catalyst 2.

Figure S33: UPLC/MS TQD spectrum and deconvoluted spectrum of photoredox catalysed modification of SUMO_M60Dha with 25 mol% water-soluble iridium photoredox catalyst 2.
S4.5 – Control and other experiments on SUMO

To prove that the designed water-soluble iridium photoredox catalyst 2 is a better catalyst for this modification than riboflavin, control experiments were performed on SUMO_G98Dha and SUMO_M60Dha in the presence of riboflavin. Catalysis was performed following the general procedure described in S4.4, but with an equal amount of riboflavin.

**Scheme S14:** General reaction scheme of photoredox catalysed modification of SUMO_G98Dha in the presence of riboflavin.

**Figure S34:** UPLC/MS TQD spectrum and deconvoluted spectrum of photoredox catalysed modification of SUMO_G98Dha with 50 mol% riboflavin.

**Figure S35:** UPLC/MS TQD spectrum and deconvoluted spectrum of photoredox catalysed modification of SUMO_G98Dha with 25 mol% riboflavin.
Scheme S15: General reaction scheme of photoredox catalysed modification of SUMO_M60Dha in the presence of riboflavin.

**Figure S36**: UPLC/MS TQD spectrum and deconvoluted spectrum of photoredox catalysed modification of SUMO_M60Dha with 50 mol% riboflavin.

**Figure S37**: UPLC/MS TQD spectrum and deconvoluted spectrum of photoredox catalysed modification of SUMO_M60Dha with 25 mol% riboflavin.
To prove that the presence of photoredox catalyst in combination with irradiation with blue LED (407.5 nm), ultimately modifies SUMO_G98Dha or SUMO_M60Dha, several control experiments were conducted. Reactions were performed with photoredox catalyst without irradiation, without any photoredox catalyst with irradiation, and without photoredox catalyst without irradiation. Catalysis was performed following the general procedure described in S4.4.

Figure S38: UPLC/MS TQD spectrum and deconvoluted spectrum of photoredox catalysed modification of SUMO_M60Dha with 25 mol% water-soluble iridium photoredox catalyst 2, but without irradiation.

Figure S39: UPLC/MS TQD spectrum and deconvoluted spectrum of photoredox catalysed modification of SUMO_G98Dha with 25 mol% water-soluble iridium photoredox catalyst 2, but without irradiation.

Figure S40: UPLC/MS TQD spectrum and deconvoluted spectrum of photoredox catalysed modification of SUMO_M60Dha without photoredox catalyst, but with irradiation.
**Figure S41**: UPLC/MS TQD spectrum and deconvoluted spectrum of photoredox catalysed modification of SUMO_G98Dha without photoredox catalyst, but with irradiation.

**Figure S42**: UPLC/MS TQD spectrum and deconvoluted spectrum of photoredox catalysed modification of SUMO_M60Dha without photoredox catalyst and without irradiation.

**Figure S43**: UPLC/MS TQD spectrum and deconvoluted spectrum of photoredox catalysed modification of SUMO_G98Dha without photoredox catalyst and without irradiation.
So far, solubility and efficiency of photoredox catalyst 2 has been proven at acidic (0.1% AcOH in H₂O, pH = 4) and neutral pH (50 mM NaH₂PO₄ 150 mM NaCl, pH = 7). To prove that photoredox catalyst 2 is also soluble and efficient at basic pH, a reaction was performed with SUMO_M60Dha in 50 mM NaH₂PO₄, pH = 8. Full conversion of SUMO_M60Dha into SUMO_M60HomoPhe was detected, showing the applicability of photoredox catalyst 2 also at basic pH.

**Scheme S16:** Reaction scheme of photoredox catalysed modification of SUMO_M60Dha at basic pH = 8.

**Figure S44:** UPLC/MS TQD spectrum and deconvoluted spectrum of photoredox catalysed modification of SUMO_M60Dha at basic pH = 8.
S5 Photoredox catalysis on the protein sfGFP

S5.1 – Stock-solution preparation for photoredox catalysis on sfGFP_L272Dha

| compound                              | molarity (mM) | solvent |
|----------------------------------------|---------------|---------|
| zinc benzylsulfinate                   | 250           | DMSO    |
| riboflavin                             | 0.5           | H₂O     |
| [Ir(dF(CF₃)ppy)₂(dNMe₃bpy)]Cl₃ (2)     | 1             | H₂O     |

*Table S7:* Stock-solutions for photoredox catalysis on sfGFP.

S5.2 – General procedure for converting cysteine to dehydroalanine

Scheme S17: General reaction scheme of the conversion of sfGFP_L272C into sfGFP_L272Dha by bis-alkylation-elimination with 2,5-dibromohexanediamide.

The cysteine containing protein sfGFP_L272C was dissolved in phosphate buffer (50 mM NaH₂PO₄, 150 mM NaCl, 50 mg/mL DTT, pH = 7.5) and diluted to a concentration of 2.5 mg/mL. An additional 1-2 mg DTT was added and the protein was incubated for 15 min. The sample was purified by PD MiniTrap™ G-25 size exclusion chromatography. 475 µL of protein solution was combined with 50 µL 2,5-dibromohexanediamide in DMF (20 mg/mL). The mixture was shaken at 750 rpm at 37 °C for 16 hours. Purification by PD MiniTrap™ G-25 size exclusion chromatography and concentration using a spinfilter (Vivaspin-15) gave the dehydroalanine containing protein sfGFP_L272Dha in full conversion as was analysed by UPLC/MS TQD.

Figure S45: UPLC/MS TQD spectrum and deconvoluted spectrum of sfGFP_L272Dha.
S5.3 – Confirmation of Dha residue by thiol Michael addition

Scheme S18: General reaction scheme of thiol Michael addition to dehydroalanine in sfGFP_L272Dha.

An aliquot of 3 nmol sfGFP_L272Dha was taken and diluted with phosphate buffer (50 mM NaH₂PO₄, 150 mM NaCl, pH 7.5) to 50 µL. 5 µL 2-Mercaptoethanol was added. The mixture was shaken at 750 rpm at 37 °C for 15 min., followed by direct analysis with UPLC/MS TQD.

Figure S46: UPLC/MS TQD spectrum and deconvoluted spectrum of thiol Michael addition to sfGFP_L272Dha.

S5.4 – General procedure of photoredox catalysis on sfGFP

Scheme S19: General reaction scheme of iridium photoredox catalysed modification of sfGFP_L272Dha.

Catalysis was performed in phosphate buffer (50 mM NaH₂PO₄, 150 mM NaCl, pH 7.5) with a final concentration of 50 µM sfGFP_L272Dha, 2.5 or 5 mM zinc benzylsulfinate, and 12.5, 25, or 50 µM iridium photoredox catalyst 2.
A typical catalysis reaction was set up as follows: To a screw-cap vial was added phosphate buffer (50 mM NaH_2PO_4, 150 mM NaCl, pH 7.5), 4 or 8 µL zinc benzylsulfinate stock-solution, and 5, 10, or 20 µL iridium photocatalyst \textbf{2} stock-solution. The solution was degassed by bubbling Ar-gas for 30 min. To the screw-cap vial was added sfGFP_L272Dha to obtain a final reaction volume of 400 µL. The reaction mixture was degassed another time by flowing Ar-gas. After 15 min., the screw-cap vial was exposed to blue LED’s for 15 min. at room temperature without stirring and subsequently loaded on a PD MiniTrap™ G-25 size exclusion chromatography column. The protein was eluted with phosphate buffer and analysed by UPLC/MS TQD.

\textbf{Figure S47:} UPLC/MS TQD spectrum and deconvoluted spectrum of photoredox catalysed modification of sfGFP_L272Dha with 25 mol% water-soluble iridium photoredox catalyst \textbf{2} and 50 eq. zinc benzylsulfinate. The spectra show recovery of the unmodified sfGFP_L272Dha.

\textbf{Figure S48:} UPLC/MS TQD spectrum and deconvoluted spectrum of photoredox catalysed modification of sfGFP_L272Dha with 50 mol% water-soluble iridium photoredox catalyst \textbf{2} and 50 eq. zinc benzylsulfinate. The spectra show recovery of the unmodified sfGFP_L272Dha.

\textbf{Figure S49:} UPLC/MS TQD spectrum and deconvoluted spectrum of photoredox catalysed modification of sfGFP_L272Dha with 50 mol% water-soluble iridium photoredox catalyst \textbf{2} and 100 eq. zinc benzylsulfinate. The spectra show generation of sfGFP_L272HomoPhe.
Figure S50: UPLC/MS TQD spectrum and deconvoluted spectrum of photoredox catalysed modification of sfGFP_L272Dha with 100 mol% water-soluble iridium photoredox catalyst 2 and 100 eq. zinc benzylsulfinate. The spectra show generation of sfGFP_L272HomoPhe.

Figure S51: UPLC/MS TQD spectrum and deconvoluted spectrum of photoredox catalysed modification of sfGFP_L272Dha with 200 mol% water-soluble iridium photoredox catalyst 2 and 100 eq. zinc benzylsulfinate (before size exclusion chromatography column). The spectra show generation of sfGFP_L272HomoPhe.
S5.5 – Control experiments sfGFP

To prove that the designed water-soluble iridium photoredox catalyst 2 is a better catalyst for this modification than riboflavin, control experiments were performed on sfGFP_L272Dha in the presence of riboflavin. Catalysis was performed following the general procedure described in S5.4, but with an equal amount of riboflavin.

Scheme S20: General reaction scheme of photoredox catalysed modification of sfGFP_L272Dha in the presence of riboflavin.

Figure S52: UPLC/MS TQD spectrum and deconvoluted spectrum of photoredox catalysed modification of sfGFP_L272Dha with 100 mol% riboflavin and 100 eq. zinc benzylsulfinate. The spectra show recovery of the unmodified sfGFP_L272Dha.

To prove that the presence of photoredox catalyst in combination with irradiation with blue LED (407.5 nm), ultimately modifies sfGFP_L272Dha, several control experiments were conducted. Reactions were performed with Ir-photoredox catalyst 2 without irradiation, without any photoredox catalyst with irradiation, and without photoredox catalyst without irradiation. Catalysis was performed following the general procedure described in section S5.4.

Figure S53: UPLC/MS TQD spectrum and deconvoluted spectrum of photoredox catalysed modification of sfGFP_L272Dha with 100 eq. zinc benzylsulfinate and 100 mol% water-soluble iridium photoredox catalyst 2, but without irradiation. The spectra show recovery of the unmodified sfGFP_L272Dha.
**Figure S54:** UPLC/MS TQD spectrum and deconvoluted spectrum of photoredox catalysed modification of sfGFP_L272Dha with 100 eq. zinc benzylsulfinate and without photoredox catalyst, but with irradiation. The spectra show recovery of the unmodified sfGFP_L272Dha.

**Figure S55:** UPLC/MS TQD spectrum and deconvoluted spectrum of photoredox catalysed modification of sfGFP_L272Dha with 100 eq. zinc benzylsulfinate, without photoredox catalyst and without irradiation. The spectra show recovery of the unmodified sfGFP_L272Dha.
S5.6 – Fluorescence measurements sfGFP

The fluorescence properties of sfGFP_L272HomoPhe are not significantly affected after modification by photoredox catalysis compared with sfGFP_L272Dha, confirmed by UV/vis absorption, emission spectroscopy and TCSPC lifetime measurements. The emission spectra, emission quantum yield (relative quantum yield is 1.04), and emission lifetime are indistinguishable, which indicates that the excited state properties of sfGFP_L272HomoPhe are not affected by the modification. The additional absorption at 400 nm for sfGFP_L272HomoPhe is probably due to some impurity that was not sufficiently removed by size-exclusion chromatography.

UV/vis absorption spectra were recorded on an AnalytikJena Specord210 UV/vis spectrometer and emission spectra on a Edinburgh Instruments FS-5 spectrofluorimeter in 1 cm pathlength quartz cuvettes. Emission spectra are corrected for instrument spectral response. Excitation was at 450 nm. Emission lifetimes were determined by TCSPC using a PLS450 pulsed diode laser, equipped with a bandpass filter and PDL800 driver for excitation and a PDM-SPAD detector connected to a PicoHarp300 standalone TCSPC module and DSN102 DualSPAD power supply from Picoquant. The SPAD (with a long pass filter 510 nm) and diode laser were coupled to a qPOD 2e temperature controlled cuvette holder from Quantum Northwest. Ludox solution was used to record instrument response functions without the 510 nm band pass filter.

**Figure S56:** Normalised UV/vis absorption spectra of sfGFP_L272Dha (in blue) and sfGFP_L272HomoPhe (in orange) in buffer.

**Figure S57:** Normalised emission spectra of sfGFP_L272Dha (in orange) and sfGFP_L272HomoPhe (in blue) in buffer. The relative emission quantum yield is 1.04.
Figure S58: Emission decay of sfGFP_L272Dha in blue ($\tau = 2.72$ ns). IRF in red.

Figure S59: Emission decay of sfGFP_L272HomoPhe in blue ($\tau = 2.60$ ns). IRF in red.
S6 Spectra

2
methyl 2-acetamidosacrylate

\[
\text{C}_6\text{H}_{12}\text{N}\text{O}_3\text{CH}_3
\]
# = zinc sulphate
* = corresponding sulphone
# = zinc sulfinate
*= corresponding sulphone
zinc 4-fluorobenzylsulfinate

# = zinc sulfate
*= corresponding sulphone
# = zinc sulfinate
* = corresponding sulphone
zinc 4-trifluoromethylbenzylsulfinate

# = zinc sulfinate
* = corresponding sulphone
# = zinc sulfinate
* = corresponding sulphone
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