Electronic Supplementary Material (ESI) for Nanoscale Horizons.

Electronic Supplementary Information

Enzyme-Inspired Flavin-Polydopamine as a Biocompatible Nanoparticle Photocatalyst

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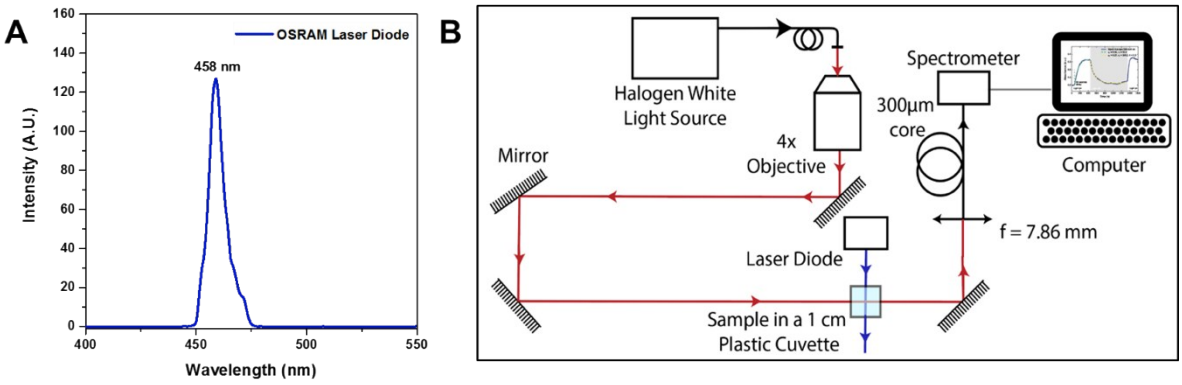
1 Materials and Methods

All materials were purchased from either Acros Organics, Alfa Aeser, Sigma-Aldrich or TCI Chemicals in the highest purity available and used without further purification.

1.1 Characterisation techniques

^{1}H and ^{13}C NMR measurements were carried out using a 500 MHz DCH Cryoprobe Spectrometer. HRMS was recorded on a ThermoFinnigan Orbitrap Classic (Fisher Scientific). UV-Vis absorption spectra were obtained with an Agilent Cary 300 Spectrophotometer. Fluorescence emission spectra were obtained using a Varian Cary Eclipse Fluorescence Spectrophotometer using excitation and emission splits of 5 nm or 10 nm. DLS and zeta potential measurements were recorded using a Zetasizer Nano Range instrument (Malvern Panalytical). FTIR spectroscopy was carried out using a Bruker Tensor 27 spectrometer with samples pressed into KBr pellets. STEM images were obtained using a Hitachi S-5500 In-Lens FE STEM (2009) at an acceleration voltage of 1.0 kV. Samples were suspended in water and drop cast on lacey carbon copper grids (Agar Scientific).



1.2 Laser Diode Emission Spectrum and Experimental Setup

Figure S1: (A) Intensity spectrum of the OSRAM laser diode used in the setup (B) Experimental Setup: The sample in a clear plastic cuvette is illuminated with a 458nm OSRAM laser diode to drive the reaction. A collimated beam from a broadband halogen white light source (Ocean Optics DH-2000) is used to measure the time dependent absorbance signal.

The time-dependent sample concentrations and reaction rates during excitation with an OSRAM laser diode (Figure S1A) were measured with a custom-built modular absorption spectroscopy setup, shown in Figure S1B. A fibre-coupled white light halogen light source (Ocean Optics DH-2000) is collimated with a 4x Olympus Plan N objective and is penetrating the sample in a clear 1x1cm plastic cuvette. The outgoing beam is coupled with a 7.86mm focal length lens into a 300muM multimode fibre, which is connected to an Ocean Optics QE65000 spectrometer. The reaction is driven with an OSRAM 458nm laser diode, spectrum shown in Figure S1A, through the cuvette perpendicular to the spectroscopy beam.

1.3 Synthesis of Flavin-Dopamine Monomer (FLDA)

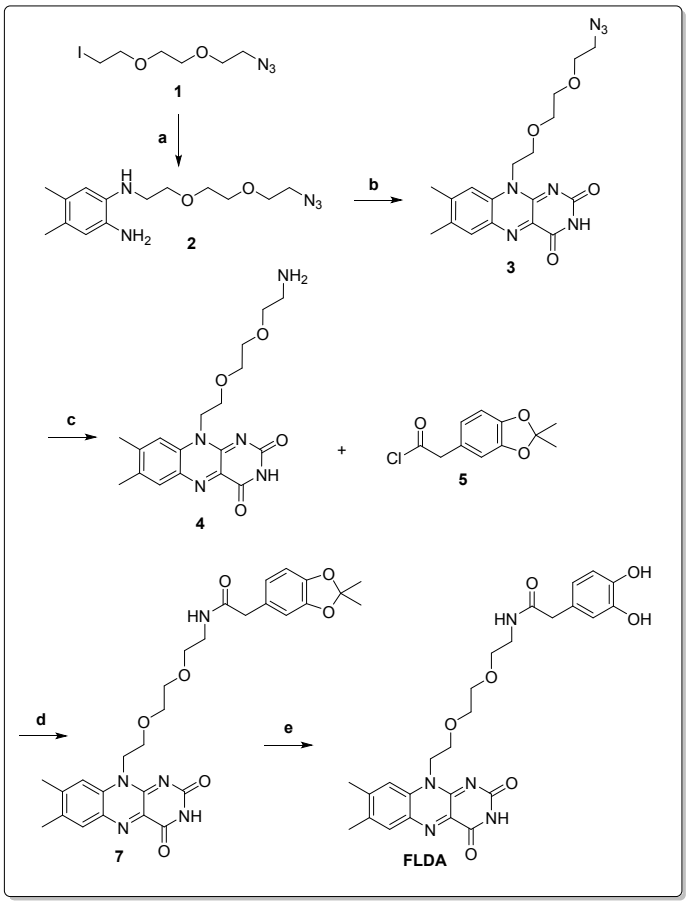
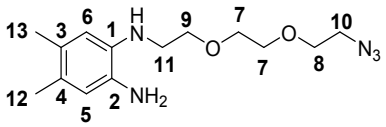


Figure S2: Reaction conditions: a) 4,5-dimethylbenzene-1,2-diamine, K_2CO_3, DMF, 50 °C, 12 h (70%); b) alloxan monohydrate, B_2O_3, AcOH, RT, dark, 48 h (69%); c) Pd/C, H_2, AcOH, RT, dark, 18 h (84%); d) TEA, DMF, RT, dark, 18 h, (62%) e) TFA/DCM, RT, dark, 2 h (99%).

***N*^{1}-(2-(2-(2-azidoethoxy)ethoxy)ethyl)-4,5-dimethylbenzene-1,2-diamine (2):**



1-Azido-2-(2-(2-iodoethoxy)ethoxy)ethane (1) was synthesised according to the literature procedure by Deng *et al.* [1]

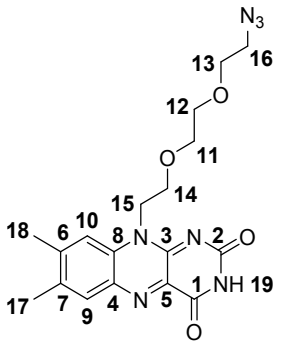
4,5-Dimethylbenzene-1,2-diamine (1.00 g, 7.34 mmol) and K_2CO_3 (2.03 g, 14.68 mmol) were dissolved in anhydrous DMF (30 mL) and heated to 50 °C under Ar atmosphere. 1-Azido-2-(2-(2-iodoethoxy)ethoxy)ethane (2.09 g, 7.34 mmol) dissolved in anhydrous DMF (10 mL) was then added dropwise and the resulting mixture was stirred at 50 °C overnight. DMF was removed under reduced pressure and the resulting residue was re-dissolved in DCM (50 mL) and washed with water (3 x 50 mL) and brine (2 x 50 mL). The organic layer was then dried over anhydrous Na_2SO_4 and evaporated. The crude product was then purified by column chromatography using the solvent system DCM/MeOH (99.5:0.5) to give the title compound as a red oil (1.50 g, 5.11 mmol, 70%).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.52 (s, 1H, **H-5**), 6.48 (s, 1H, **H-6**), 3.74 (t, 2H, ^3J = 5.2 Hz, **H-9**), 3.70-3.67 (m, 6H, **H-7**, **H-9**), 3.39 (t, 2H, ^3J = 5.1 Hz, **H-10**), 3.27 (br. s, 2H, **H-11**), 2.15 (2 x br. s, 6H, **H-12**, **H-13**) ppm

^{13}C NMR (500 MHz, $CDCl_3$): δ = 134.91 (**C-1**), 132.93 (**C-2**), 127.72 (**C-3**), 126.90 (**C-4**), 118.24 (**C-5**), 115.11 (**C-6**), 70.68 (**C-7**), 70.35 (**C-8**), 70.11 (**C-9**), 50.71 (**C-10**), 44.45 (**C-11**), 19.24 (**C-12**), 18.84 (**C-13**) ppm

HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{14}H_{24}O_2N_5$ 294.1925; Found 294.1914.

10-(2-(2-(2-Azidoethoxy)ethoxy)ethyl)-7,8-dimethylbenzo[g]pteridine-2,4(3H,10H)-dione (3):



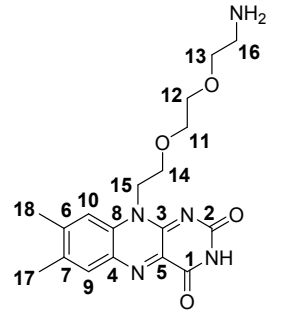
N^1-(2-(2-(2-azidoethoxy)ethoxy)ethyl)-4,5-dimethylbenzene-1,2-diamine (2.50 g, 8.52 mmol), B_2O_3 (1.19 g, 17.04 mmol) and alloxan monohydrate (1.21 g, 8.52 mmol) were dissolved in glacial acetic acid (25 mL) and left to stir in the dark at room temperature under Ar atmosphere for 2 days. Water was added (25 mL) and extracted with DCM (3 x 50 mL). The organic layer was evaporated and then co-evaporated with toluene (3 x 50 mL) to remove any traces of water and acetic acid. The crude product was purified by column chromatography using a gradient solvent system of DCM/acetone (4:1 - 1:1) to give the title compound as an orange solid (2.35 g, 5.89 mmol, 69%)

^{1}H NMR (500 MHz, DMSO-d_6): δ =11.30 (s, 1H, **H-19**), 7.82 (s, 1H, **H-9**), 7.81 (s, 1H, **H-10**), 4.76 (t, 2H, ^3J = 5.8 Hz, **H-15**), 3.81 (t, 2H, ^3J = 5.8 Hz, **H-14**), 3.54 (m, 2H, **H-13**), 3.45 (m, 4H, **H-11**, **H-12**), 3.25 (t, 2H, ^3J = 5.8 Hz, **H-16**), 2.47 (s, 1H, **H-17**), 2.36 (s 1H, **H-18**) ppm

^{13}C NMR (500 MHz, DMSO-d_6): δ = 160.33 (**C-1**), 155.98 (**C-2**), 150.66 (**C-3**), 146.65 (**C-4**), 137.48 (**C-5**), 136.20 (**C-6**), 134.10 (**C-7**), 131.83 (**C-8**), 131.18 (**C-9**), 117.30 (**C-10**), 70.67 (**C-11**), 70.06 (**C-12**), 69.73 (**C-13**), 67.24 (**C-14**), 50.39 (**C-15**), 44.56 (**C-16**), 21.06 (**C-17**), 19.22 (**C-18**) ppm

HRMS (ESI) m/z: $[M + Na]^+$ Calcd for $C_{18}H_{21}O_4N_7Na$ 422.1547; Found 422.1528.

10-(2-(2-(2-aminoethoxy)ethoxy)ethyl)-7,8-dimethylbenzo[g]pteridine-2,4(3H,10H)-dione (4):



10-(2-(2-(2-Azidoethoxy)ethoxy)ethyl)-7,8-dimethylbenzo[g]pteridine-2,4(3H,10H)-dione (0.50 g, 1.25 mmol) was added to a dried 100 mL 3 neck round bottom flask and dissolved in degassed glacial acetic acid (50 mL). The vessel was purged with Ar by water aspiration before adding Pd/C (10 mg), rinsing any residual powder off of the flask walls with degassed acetic acid. The vessel was purged with Ar again before replacing the atmosphere with H_2 from a balloon. The reaction was then left to stir for 18 h at room temperature. The atmosphere was then replaced by Ar and the reaction mixture filtered through celite, washed with methanol and evaporated. The crude residue was subjected to

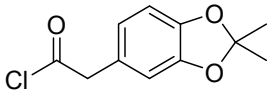
flash column chromatography using the solvent system DCM/MeOH/AcOH (70:20:10 - 20:70:10) to give the title compound as a red residue (0.43 g, 1.05 mmol, 84%).

^{1}H NMR (500 MHz, CD_3OD): δ = 8.20 (s, 1H, **H-9**), 8.14 (s, 1H, **H-10**), 5.17 (t, 2H, ^{3}J = 5.0 Hz, **H-15**), 4.13 (t, 2H, ^{3}J = 5.0 Hz, **H-14**), 3.68 (m, 2H, **H-13**), 3.64 – 3.59 (m, 4H, **H-11**, **H-12**), 3.08 (t, 2H, ^{3}J = 5.0 Hz, **H-16**), 2.68 (s, 3H, **H-17**), 2.55 (s, 3H, **H-18**) ppm

^{13}C NMR (500 MHz, CD_3OD): δ = 158.9 (**C-1**), 151.6 (**C-2**), 150.8 (**C-3**), 145.9 (**C-4**), 140.6 (**C-5**), 137.1 (**C-6**), 134.4 (**C-7**), 131.3 (**C-8**), 130.5 (**C-9**), 117.3 (**C-10**), 70.4 (**C-11**), 69.8 (**C-12**), 67.7 (**C-13**), 66.4 (**C-14**), 42.8 (**C-15**), 39.2 (**C-16**), 20.3 (**C-17**), 18.2 (**C-18**) ppm

HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{18}H_{24}O_4N_5$ 374.1823; Found 374.1828.

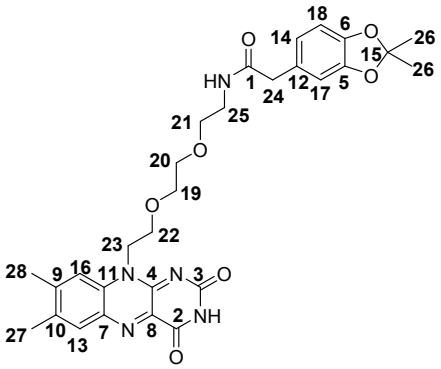
2-(2,2-Dimethylbenzo[d][1,3]dioxol-5-yl)acetyl chloride (5):



2-(2,2-Dimethylbenzo[d][1,3]dioxol-5-yl)acetic acid was synthesised according to the literature procedure by Geiseler *et al.*[2]

2-(2,2-Dimethylbenzo[d][1,3]dioxol-5-yl)acetic acid (0.42 g, 2.02 mmol) was dissolved in anhydrous DCM (40 mL) before adding $SOCl_2$ (1.46 mL, 20.17 mmol) and refluxing for 2 h under Ar atmosphere. The reaction mixture was evaporated to remove the solvent and excess $SOCl_2$ was removed by toluene co-evaporation (3 x 20 mL). The resulting dark brown oil was immediately used in the following step without further purification (0.46 g, 2.02 mmol, 100%)

N-(2-(2-(2-(7,8-Dimethyl-2,4-dioxo-3,4-dihydrobenzo[g]pteridin-10(2H)-yl)ethoxy)ethoxy)ethyl)-2-(2,2-dimethylbenzo[d][1,3]dioxol-5-yl)acetamide (6):



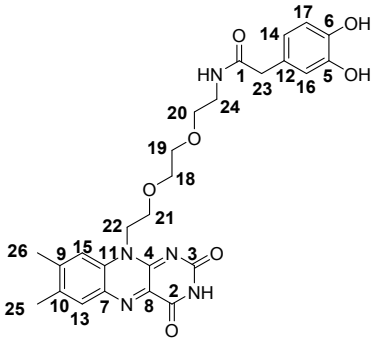
10-(2-(2-(2-aminoethoxy)ethoxy)ethyl)-7,8-dimethylbenzo[g]pteridine-2,4(3H,10H)-dione (0.40 g, 0.98 mmol) was dissolved in anhydrous DMF (40 mL) under Ar atmosphere and protected from light. Et_3N (0.27 mL, 1.95 mmol) was added and the solution turned form dark red to dark green. The mixture was then cooled to 0 °C before adding 2-(2,2-dimethylbenzo[d][1,3]dioxol-5-yl)acetyl chloride (0.44 g, 1.95 mmol) dissolved in anhydrous DMF (10 mL) dropwise over 20 minutes. The resulting dark red reaction mixture was left to stir and warm to room temperature over 18 h. The solvent was then removed and the crude residue purified by column chromatography using the solvent system DCM/MeOH/AcOH (98.5:1:0.5 – 90:6:4) to give the title compound as an orange solid (0.355 g, 0.63 mmol, 64%).

^{1}H NMR (500 MHz, CD_3OD): δ = 7.94 (s, 1H, **H-13**), 7.91 (s, 1H, **H-16**), 6.64 (m, 2H, **H-17**, **H-18**), 6.59 (d, 1H, ^{3}J = 8.1 Hz, **H-14**), 4.97 (t, 2H, ^{3}J = 5.4 Hz, **H-23**), 3.98 (t, 2H, ^{3}J = 5.4 Hz, **H-22**), 3.58 (m, 2H, **H-19**), 3.46 (m, 2H, **H-20**), 3.36 (t, 2H, ^{3}J = 5.6 Hz, **H-21**), 3.34 (s, 2H, **H-24**) 3.21 (t, 2H, ^{3}J = 5.6 Hz, **H-25**), 2.56 (s, 3H, **H-27**), 2.46 (s, 3H, **H-28**), 1.58 (s, 6H, **H-26**) ppm

^{13}C NMR (500 MHz, CD_3OD): δ = 173.0 (**C-1**), 157.8 (**C-2**), 150.6 (**C-3**), 147.8 (**C-4**), 147.5 (**C-5**), 146.4 (**C-6**), 137.5 (**C-7**), 137.2 (**C-8**), 136.2 (**C-9**), 135.7 (**C-10**), 134.8 (**C-11**), 132.2 (**C-12**), 130.9 (**C-13**), 121.3 (**C-14**), 117.6 (**C-15**), 117.1 (**C-16**), 108.7 (**C-17**), 107.5 (**C-18**), 70.5 (**C-19**), 69.9 (**C-20**), 69.0 (**C-21**), 67.5 (**C-22**), 45.0 (**C-23**), 42.1 (**C-24**), 39.0 (**C-25**), 24.5 (**C-26**), 20.0 (**C-27**), 18.0 (**C-28**) ppm

HRMS (ESI) m/z: [M + H]+ Calcd for $C_{29}H_{34}O_7N_5$ 564.2453; Found 564.2448.

2-(3,4-Dihydroxyphenyl)-N-(2-(2-(2-(7,8-dimethyl-2,4-dioxo-3,4-dihydrobenzo[g]pteridin-10(2H)-yl)ethoxy)ethoxy)ethyl)acetamide (FLDA):



N-(2-(2-(2-(7,8-Dimethyl-2,4-dioxo-3,4-dihydrobenzo[g]pteridin-10(2H)-yl)ethoxy)ethoxy)ethyl)-2-(2,2-dimethylbenzo[d][1,3]dioxol-5-yl)acetamide (0.20 g, 0.35 mmol) was dissolved in DCM (20 mL) and TFA (4 mL) was added dropwise at 0 °C. The reaction mixture was then left to stir at room temperature until TLC analysis showed completion (2 h). The solvent and excess TFA were removed under reduced pressure and co-evaporated with toluene (3 x 20 mL) to give the title compound as a red solid (0.184 g, 0.35 mmol, 99%)

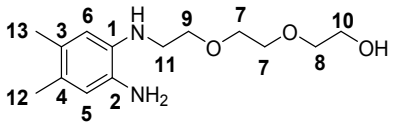
^{1}H NMR (500 MHz, CD_3OD): δ = 7.84 (s, 1H, **H-13**), 7.82 (s, 1H, **H-15**), 6.67 (d, 1H, ^4J = 2.1 Hz, **H-16**), 6.64 (d, 1H, ^3J = 8.0 Hz, **H-17**), 6.54 (dd, 1H, ^3J = 8.0 Hz, ^4J = 2.1 Hz, **H-14**), 4.90 (t, 2H, ^3J = 5.5 Hz, **H-22**), 3.94 (t, 2H, ^3J = 5.5 Hz, **H-21**), 3.55 (m, 2H, **H-18**), 3.44 (m, 2H, **H-19**), 3.36 (t, 2H, ^3J = 5.5 Hz, **H-20**), 3.29 (s, 2H, **H-23**) 3.21 (t, 2H, ^3J = 5.5 Hz, **H-24**), 2.52 (s, 3H, **H-25**), 2.42 (s, 3H, **H-26**) ppm

^{13}C NMR (500 MHz, CD_3OD): δ = 174.8 (**C-1**), 162.3 (**C-2**), 151.7 (**C-3**), 149.4 (**C-4**), 146.4 (**C-5**), 145.3 (**C-6**), 138.9 (**C-7**), 138.7 (**C-8**), 137.3 (**C-9**), 136.1 (**C-10**), 133.5 (**C-11**), 132.2 (**C-12**), 128.2 (**C-13**), 121.5 (**C-14**), 118.5 (**C-15**), 117.2 (**C-16**), 116.4 (**C-17**), 71.9 (**C-18**), 71.3 (**C-19**), 70.5 (**C-20**), 68.8 (**C-21**), 46.4 (**C-22**), 43.3 (**C-23**), 40.4 (**C-24**), 21.4 (**C-25**), 19.4 (**C-26**) ppm

HRMS (ESI) m/z: [M + H]+ Calcd for $C_{26}H_{29}O_7N_5Na$ 546.1959; Found 546.1948.

1.5 Synthesis of DOPAC-free flavin (FLOH)

2-(2-(2-((2-amino-4,5-dimethylphenyl)amino)ethoxy)ethoxy)ethan-1-ol:



4,5-dimethylbenzene-1,2-diamine (1.41 g, 10.32 mmol) and TEA (2.40 mL, 17.20 mmol) were dissolved in dry THF (40 mL) and heated to 60°C before adding an excess of NaI (5.16 g, 34.40 mmol) and the dropwise addition of 2-(2-(2-chloroethoxy)ethoxy)ethan-1-ol (1.00 mL, 6.88 mmol) dissolved in THF (5 mL). The resulting heterogeneous mixture was then stirred overnight under inert atmosphere at 60°C. After cooling, the mixture was diluted with water (100 mL) and extracted with DCM (5 x 50 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and reduced under reduced pressure. The

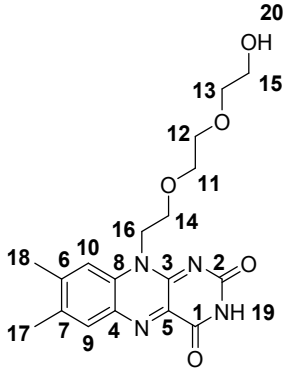
resulting dark red residue was purified via silica gel column chromatography in DCM-methanol (98:2) to yield a red oil (1.242 g, 4.63 mmol, 67%).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.52 (s, 1H, **H-5**), 6.47 (s, 1H, **H-6**), 3.76 (t, 2H, ^{3}J = 5.1 Hz, **H-10**), 3.70 (t, 2H, ^{3}J = 5.1 Hz **H-9**), 3.67 (br. s, 4H, **H-7**), 3.59 (m, 2H, **H-8**), 3.26 (t, 2H, ^{3}J = 5.1 Hz, **H-11**), 2.16 (s, 3H, **H-12**), 2.12 (s, 3H, **H-13**) ppm

^{13}C NMR (500 MHz, $CDCl_3$): δ = 135.15 (**C-1**), 132.44 (**C-2**), 128.01 (**C-3**), 126.71 (**C-4**), 118.45 (**C-5**), 114.82 (**C-6**), 72.79 (**C-7**), 70.36 (**C-7**), 70.00 (**C-8**), 69.63 (**C-9**) 50.71 (**C-10**), 44.2 (**C-11**), 19.27 (**C-12**), 18.82 (**C-13**) ppm

HRMS (ESI) m/z: [M + H]+ Calcd for $C_{14}H_{24}O_3N_2Na$ 291.167914; Found 291.16703.

10-(2-(2-(2-hydroxyethoxy)ethoxy)ethyl)-7,8-dimethylbenzo[g]pteridine-2,4(3H,10H)-dione (FLOH):



2-(2-(2-((2-amino-4,5-dimethylphenyl)amino)ethoxy)ethoxy)ethan-1-ol (1.50 g, 5.59 mmol), B_2O_3 (0.778 g, 11.18 mmol) and alloxan monohydrate (0.894 g, 5.59 mmol) were dissolved in glacial acetic acid (17 mL) and left to stir in the dark at room temperature under Ar atmosphere for 2 days. Water was added (25 mL) and the mixture extracted with DCM (3 x 50 mL). The organic layer was evaporated and then co-evaporated with toluene (3 x 50 mL) to remove any traces of water and acetic acid. The crude product was purified by column chromatography using a gradient solvent system of DCM/acetone (4:1 - 1:1) to give the title compound as a yellow solid (1.49 g, 3.98 mmol, 71%)

^{1}H NMR (500 MHz, DMSO-d_6): δ =11.19 (s, 1H, **H-19**), 7.84 (s, 1H, **H-9**), 7.82 (s, 1H, **H-10**), 4.79 (t, 2H, ^{3}J = 5.9 Hz, **H-15**), 4.40 (t, 1H, ^{3}J = 5.5 Hz, **H-20**), 3.85 (t, 2H, ^{3}J = 5.9 Hz, **H-14**), 3.56 (t, 2H, ^{3}J = 5.9 Hz, **H-13**), 3.45 (m, 4H, **H-11**, **H-12**), 3.34 (m, 2H, **H-16**), 2.47 (s, 1H, **H-17**), 2.36 (s 1H, **H-18**) ppm

^{13}C NMR (500 MHz, DMSO-d_6): δ = 160.12 (**C-1**), 155.76 (**C-2**), 150.58 (**C-3**), 146.56 (**C-4**), 137.34 (**C-5**), 136.05 (**C-6**), 134.05 (**C-7**), 131.74 (**C-8**), 131.14 (**C-9**), 117.18 (**C-10**), 72.74 (**C-11**), 70.59 (**C-12**), 70.10 (**C-13**), 67.13 (**C-14**), 60.60 (**C-15**), 44.56 (**C-16**), 20.91 (**C-17**), 19.05 (**C-18**) ppm

HRMS (ESI) m/z: $[M + Na]^+$ Calcd for $C_{18}H_{22}O_5N_4Na$ 397.1482; Found 397.1467.

1.5 Synthesis of Flavin-Polydopamine

General Procedure: A mixture of ammonia solution (0.1 mL, 28%), ethanol (1.5 mL) and Milli Q water (4.5 mL) was stirred at room temperature for 30 minutes in reaction vessels protected from direct sunlight. Dopamine hydrochloride* dissolved in Milli-Q water (0.5 mL) and FLDA** dissolved in ethanol (0.5 mL) were mixed before being added dropwise to the reaction mixture. The resulting dark brown/black mixture was left to stir in the presence of air for 24 h. The mixture was then centrifuged at 25000 x *g* for 30 min and the supernatant was removed. The precipitate was washed with Milli-Q water (3 x 40 mL) and then suspended in Milli-Q water (20 mL), frozen in liquid N_2 and lyophilised to yield a dark brown/black powder.

Sample	*Dopamine Hydrochloride / mg	**FLDA / mg	Molar Ratio (DA.HCl:FLDA)
FLPDA- 5	15.80	8.73	5:1
FLPDA-10	17.24	4.76	10:1
FLPDA-20	18.06	2.49	20:1
PDA	18.96	-	-

1.6 Photooxidation of Amplex Red

General Procedure: **FLPDA** catalyst (1-10 µg/mL) and Amplex Red (100 µM) were added to a cuvette containing KP_i buffer (2 mL, 10 mM, pH 7.4) with stirring in the dark. The cuvette was then irradiated with a laser diode (458 nm) and spectra were recorded over time. Absorbance values were converted to molar equivalents using the molar extinction coefficient of resorufin at pH 7.4 (67 700 $M^{-1}cm^{-1}$).[3]

1.7 Photoreduction of Resazurin

General Procedure: **FLPDA** catalyst (1-10 µg/mL), EDTA (100 µM) and resazurin (25 µM) were added to a cuvette containing KP_i buffer (2 mL, 10 mM, pH 7.4) with stirring. The mixture was purged with N_2 in the dark for 15 minutes. It was then irradiated with a laser diode (458 nm) under N_2 atmosphere and spectra were recorded over time. Absorbance values were converted to molar equivalents using the molar extinction coefficient of resorufin at 572 nm (67 700 $M^{-1}cm^{-1}$, pH 7.4)[3] and for resazurin at 602 nm (35 510 $M^{-1}cm^{-1}$, pH 7.4) which was calculated using known concentrations of resazurin.

1.8 Cell Culture

A549 cells were cultured within 25, 75 or 175 cm^2 flasks in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10 % (v/v) sterile filtrated (0.2 µM CA membrane filter) fetal bovine serum (FBS), 100 U/mL penicillin and 100 µg/mL streptomycin sulphate (PS) at 37 °C and 5 % CO_2 in a humidified incubator and sub-cultured at 80 % confluency. For passaging cells, the medium was removed, cells were washed with 2-5 mL Dulbecco's Phosphate buffered saline (PBS) before incubating the adherent cells for 5 min with 2-5 mL trypsin-EDTA solution (0.25 % v/v) in order to detach the adherent cells from the culture flask. After stopping trypsinization by adding at least the same volume of DMEM, the cells were separated from the medium by centrifugation (5 min, 100 x *g,* RT). Subsequently, the cell pellet was resuspended and diluted in DMEM according to the desired size and cell density of the following cell passage (1:4 – 1:8).

To adjust a cell suspension to a specific cell number, 20 µL of the homogenously resuspended cell pellet were mixed with 20 µL of Trypan Blue, before counting the available cells in a volume of 0.1 mm^3 with a haemocytometer (0.1 mm depth) and diluting the cell suspension to the desired cell density.

1.8.1 LDH release cytotoxicity assay

To evaluate the *in vitro* cytotoxicity of **FLPDA** nanoparticles on A549 cells, the activity of the cytosolic enzyme lactate dehydrogenase (LDH) present in the culture media was determined by using the commercially available fluorometric LDH assay kit in a 96-well plate format (abcam, UK). Hereby, released LDH catalysed the oxidation of lactate to pyruvate with simultaneous reduction of NAD^+ to NADH. Subsequently, an assay kit specific reactant oxidized NADH to NAD^+ and thereby was reduced itself, which leads to a fluorescent product with excitation at 535 nm and emission at 587 nm. Thus, the rate of increasing fluorescence is directly proportional to the LDH activity in the cell medium and thereby to the number of damaged cells.

Prior to the dose-response cytotoxicity assay, different parameters of the assay were optimized to receive a reliable readout. As the amount of LDH-processed NADH is only proportional to the measured fluorescence within a certain linear range, determined by the technical specifications of the used fluorimeter, the initially seeded cell number and the volume of analysed culture media has to be adapted to ensure a readout within this range.

Therefore, either 4 x 10^3, 1 x 10^4 or 8 x 10^4 cells per well were seeded in a total volume of 100 µL DMEM supplemented with 10 % FBS (v/v), 100 U/mL penicillin and 100 µg/mL streptomycin sulphate within a clear, flat-bottom 96-well plate and cultured for 24 hours at 37 °C, 5 % CO_2. Subsequently, the cells were exposed to either DMEM supplemented with 0.25 % FBS (v/v), 100 U/mL penicillin and 100 µg/mL streptomycin sulphate (negative treatment control) or to the aforementioned medium but additionally supplemented with 0.1 % (v/v) Triton X-100 (positive treatment control). After further 24 hours of incubation at 37 °C, 5 % CO_2, different volumes of the culture media (50 µL, 10 µL or 5 µL) were analysed using the fluorimetric LDH assay kit, which was performed in accordance to the manufacturer´s instructions. For the fluorometric assay, 5 µL of culture media were transferred to a new, black 96-well plate and supplemented with 95 µL of reaction mix before incubating the plate for 10 min at room temperature and measuring the fluorescence at Ex/Em = 535/587 nm (Tecan Spark®, Tecan). Each condition, involving the cell-free assay negative control (LDH assay buffer) and the cell-free assay positive control (provided LDH enzyme), was tested in biological triplicates. For the analysis, all readings were corrected by subtracting the mean fluorescence of the blank sample (assay negative control) before averaging all replicate readings.

To determine the dose-response effects of the nanoparticles, the respective cell-line specific optimized cell number (A549: 1*10^4 cells/well) was seeded like described above within a transparent 96-well plate. Subsequently, the medium of the cells was replaced by new medium (DMEM, 0.25 % FBS (v/v), 100 U/mL penicillin, 100 µg/mL streptomycin sulphate) supplemented with different concentrations of nanoparticles (1.0 mg/mL, 0.1 mg/mL, 10.0 µg/mL, 1.0 µg/mL, 0.1 µg/mL, 10.0 ng/mL, 1.0 ng/mL or 0.1 ng/mL), 0.1 % (v/v) Triton X-100 (positive treatment control) or without supplements (negative treatment control), before incubating the cells for further 24 h at 37 °C. Afterwards, the previously optimized volume of the culture media was analysed using the fluorometric LDH assay kit like described above. Each condition, involving the cell-free assay negative control (LDH assay buffer) and the cell-free assay positive control (provided LDH enzyme), was tested in biological triplicates.

For analysis, all readings were corrected by subtracting the mean fluorescence of the blank sample (assay negative control) before averaging all replicate readings. Subsequently, the percentage of cell cytotoxicity was determined as fluorescence ratio between the test sample (particular concentration of nanoparticle) and the maximum signal from the lysed cell control, which both were previously corrected by the minimum signal from the untreated cell control (see Equation (1)). The percentage of cell cytotoxicity was plotted against the applied concentration of nanoparticles.

$$Cell\ Cytotoxicity\ (\%) = \left(\frac{(Test\ sample - \ Untreated\ cell\ control)}{(Lysed\ cell\ control - Untreated\ cell\ control)} \right) x\ 100 \quad \textbf{(1)}$$

The potential interference of nanoparticles with the LDH assay was tested in a cell-free system for the assay negative control (to test whether particles may catalyse the reaction in absence of LDH or interfere with the fluorescence reading), as well as for the assay positive control by incubating provided LDH with the nanoparticles (to test whether particles inhibit LDH enzyme or reveal quenching effects). The interference test was performed with a nanoparticle concentration of 0.1 mg/mL for a duration of 30 min.

1.8.2 MTS viability assay

To evaluate the *in vitro* viability of A549 cells with nanoparticle treatment, the metabolic activity of the cells was determined by using the commercially available MTS viability assay kit in a 96-well plate format (Promega, USA). Hereby, the tetrazolium compound MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) is presumably bioreduced by NADPH or NADH (produced by dehydrogenase enzymes in metabolic active cells) into a soluble, coloured formazan product, which can be colorimetrically detected between 450-540 nm. Thus, the measured absorbance is directly proportional to the amount of metabolic active cells in culture. Because the negatively charged MTS cannot readily penetrate the cells, the included electron acceptor reagent PES (phenazine ethyl sulfate) transfers the electrons from the cytoplasm to the MTS in the media and thereby facilitates its reduction.[4]

The establishment of the MTS assay requires the optimization of the initially seeded cell number, the incubation time of the MTS solution as well as the wavelength of the spectroscopic analysis. Therefore, either 4 x 10^3, 1 x 10^4, 5 x 10^4 or 8 x 10^4 cells per well were seeded in a total volume of 100 µL DMEM supplemented with 10 % FBS (v/v), 100 U/mL penicillin and 100 µg/mL streptomycin sulphate within a transparent 96-well plate and cultured for 24 hours at 37 °C, 5 % CO_2. Subsequently, the cells were exposed to either DMEM supplemented with 0.25 % FBS (v/v), 100 U/mL penicillin and 100 µg/mL streptomycin sulphate (positive control) or to the aforementioned medium but additionally supplemented with 0.1 % (v/v) Triton X-100 (negative control). After further 24 hours of incubation at 37 °C, 5 % CO_2, the media was replaced by new media and cell viability was analysed using the MTS assay kit, which was performed in accordance to the manufacturer´s instructions. Therefore, 20 µL of the MTS solution were added to each well before incubating the plate for 1 - 4 hours at 37 °C, 5 % CO_2 while measuring the absorbance at 490 nm, 520 nm and 540 nm every hour (SPECTROstar Nano, BMG Labtech). Each condition, involving the cell-free assay negative control (culture medium), was tested in biological triplicates.

For the analysis, all readings were corrected by subtracting the mean absorbance of the blank sample (assay negative control) before averaging all replicate readings. Subsequently, all averaged replicate readings were compared to the technical specifications of the used UV-Vis spectrophotometer (SPECTROstar Nano, BMG Labtech), which declared an accuracy of <1 % and a precision of < 0.8 % at 2 OD. Thus, the assay parameters were adjusted so that the corresponding absorbance values do not significantly exceed 2 OD.

To determine the dose-response effects of a particular nanoparticle, the respective cell-line specific optimized cell number (A549: 4*10^3 cells/well) was seeded like described above within a transparent 96-well plate. Subsequently, the medium of the cells was replaced by new medium (DMEM, 0.25 % FBS (v/v), 100 U/mL penicillin, 100 µg/mL streptomycin sulphate) supplemented with different concentrations of the nanoparticles (1.0 mg/mL, 0.1 mg/mL, 10.0 µg/mL, 1.0 µg/mL, 0.1 µg/mL, 10.0 ng/mL, 1.0 ng/mL or 0.1 ng/mL), 0.1 % (v/v) Triton X-100 (negative control) or without supplements (positive control), before incubating the cells for further 24 h at 37 °C. Afterwards, the media was again replaced and 20 µL of the MTS solution were added to each well before incubating the plate for

2 hours at 37 °C, 5 % CO_2 and measuring the absorbance at 540 nm (SPECTROstar Nano, BMG Labtech). Each condition, involving the cell-free assay negative control (culture medium), was tested in biological triplicates.

For analysis, all readings were corrected by subtracting the mean absorbance of the blank sample (assay negative control) before averaging all replicate readings. Subsequently, the percentage of cell viability was determined as absorbance ratio between the test sample (particular concentration of nanoparticle) and the maximum signal from the untreated cell control, which both were previously corrected by the minimum signal from the lysed cell control (see Equation (2)). The percentage of cell viability was plotted against the applied concentration of nanoparticles.

$$Cell\ Viability\ (\%) = \left(\frac{(Test\ sample - \ Lysed\ cell\ control)}{(Untreated\ cell\ control - Lysed\ cell\ control)} \right) x\ 100 \quad \textbf{(2)}$$

Potential interferences of the nanoparticles with the MTS assay were previously tested in a cell-free system for the assay negative control (to test whether particles may catalyse the detection reaction in absence of cells), as well as for the cell-containing positive control by adding the nanoparticles to viable cells (cultured without nanoparticles) shortly before adding the MTS solution (to test whether the detection reaction is influenced by the nanoparticles). The interference test was performed with a nanoparticle concentration of 0.1 µg/mL for a duration of 4 hours before absorbance was measured at 490 nm, 520 nm and 540 nm.

1.8.2 Superoxide detection assay

To evaluate the level of oxidative stress for A549 cells with nanoparticle treatment, the amount of intracellular ROS was detected using the commercially available superoxide detection kit was used in a 96-well plate format (Enzo Life Sciences Ltd., UK), which however, only detects superoxide as one representative of the ROS family. Hereby, a cell-permeable probe reacts specifically with superoxide ions (O_2^-), generating an orange fluorescent product, which can be detected by fluorescence measurements at an excitation of 550 nm and an emission at 620 nm. Thus, the measured fluorescence intensity is directly proportional to the amount of superoxide species in the cultured cells.

The establishment of the ROS detection assay requires the optimization of the initially seeded cell number. Therefore, either 1 x 10^3, 4 x 10^3, 1 x 10^4, 5 x 10^4 or 8 x 10^4 cells per well were seeded in a total volume of 100 µL colourless DMEM without phenol red but supplemented with 10 % FBS (v/v), 100 U/mL penicillin and 100 µg/mL streptomycin sulphate within a transparent 96-well plate and cultured for 24 hours at 37 °C, 5 % CO_2. Subsequently, the cells were exposed to colourless DMEM, supplemented with 0.25 % FBS (v/v), 100 U/mL penicillin and 100 µg/mL streptomycin sulphate and incubated for further 24 hours at 37 °C, 5 % CO_2 before analysing the presence of superoxide species using the superoxide detection assay kit, which was performed in accordance to the manufacturer´s instructions. Hereby, the cells were washed once in 1 x wash buffer and subsequently incubated with 1 µM superoxide detection reagent in 1 x wash buffer for 60 minutes at 37 °C, during which the cells of the control samples were treated by either inducing the cellular ROS production through the addition of 200 µM ROS-inducer pyocyanin (positive control) or without further cell treatment (untreated control). Subsequently, the presence of detectable fluorescent product was directly measured at Ex/Em = 550/620 nm (Tecan Spark®, Tecan). Each condition, involving the cell-free assay background control (1x wash buffer), was tested in biological triplicates. For the analysis, all readings were corrected by subtracting the mean fluorescence of the blank sample (assay background control) before averaging all replicate readings.

To determine the dose-response effects of the nanoparticles, the respective cell-line specific optimized cell number (A549: 1*10^4 cells/well) was seeded like described above within a black 96-well

plate. Subsequently, the medium of the cells was replaced by new medium (colourless DMEM, 0.25 % FBS (v/v), 100 U/mL penicillin, 100 µg/mL streptomycin sulphate) supplemented with different concentrations of the nanoparticles (1 mg/mL, 100 µg/mL, 10 µg/mL, 1 µg/mL, 100 ng/mL, 10 ng/mL, 1 ng/mL or 0.1 ng/mL) or without supplements (positive and negative treatment control), before incubating the cells for further 23 h at 37 °C. Afterwards, the cells were analysed with the superoxide detection assay kit like described above and fluorescence was measured at Ex/Em = 550/620 nm (Tecan Spark®, Tecan). Each condition, involving the cell-free assay negative control (1x wash buffer), was tested in biological triplicates.

For analysis, all readings were corrected by subtracting the mean absorbance of the blank sample (assay negative control) before averaging all replicate readings. Subsequently, the percentage of ROS induction was determined as fluorescence ratio between the test sample (particular concentration of nanoparticle) and the maximum signal from the ROS-induced cell control, which both were previously corrected by the minimum signal from the untreated cell control (see Equation (3)). The percentage of ROS-induction was plotted against the applied concentration of nanoparticles.

$$Superoxide\ Induction\ (\%) = \left(\frac{(Test\ sample - \ Untreated\ cell\ control)}{(ROS\text{-}induced\ sample - Untreated\ cell\ control)} \right) x\ 100 \quad \textbf{(3)}$$

The potential interference of nanoparticles with the superoxide detection assay was previously tested in a cell-free system for the assay negative control (to test whether particles may catalyse the detection reaction in absence of cells), as well as for the cell-containing positive control by adding the nanoparticles to untreated cells (cultured without nanoparticles) 30 minutes before adding the ROS-inducer (to test whether the detection reaction is influenced by the nanoparticles). The interference test was performed with a nanoparticle concentration of 0.1 mg/mL for a duration of 60 minutes (recommended), 70 minutes or 80 minutes before fluorescence was measured like described above.

2 Supplementary Data

2.1 DLS and Zeta Potential Measurements

Table S1: DLS and zeta potential measurements of FLPDA samples. Errors are the standard deviation of the triplicate data. Sizes from STEM images were determined from the mean of >100 measurements of spherical particles, with the associated error being the standard deviation.

Sample	Monomer molar ratio (DA:FLDA)	Hydrodynamic size / nm	PDI	Zeta potential / mV	Size (STEM) / nm
FLPDA-5	5:1	577.3 ± 37.32	0.448	-35.7 ± 0.3	202 ± 110
FLPDA-10	10:1	495.5 ± 23.05	0.382	-37.7 ± 0.8	207 ± 53
FLPDA-20	20:1	389.4 ± 16.83	0.334	-41.5 ± 0.8	201 ± 58
PDA	1:0	155.0 ± 1.12	0.044	-55.0 ± 1.2	110 ± 18

2.2 STEM Images

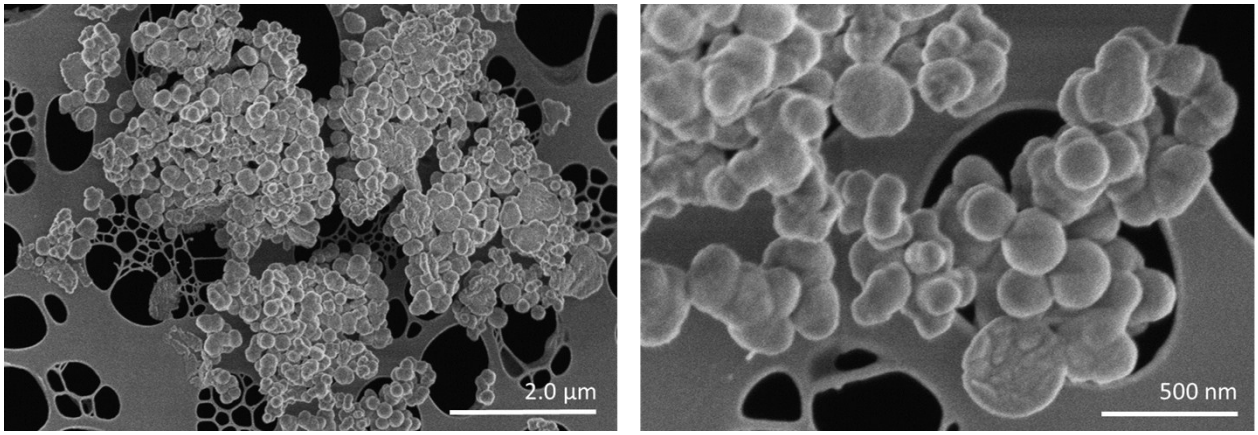


Figure S3: STEM images of **FLPDA-20**

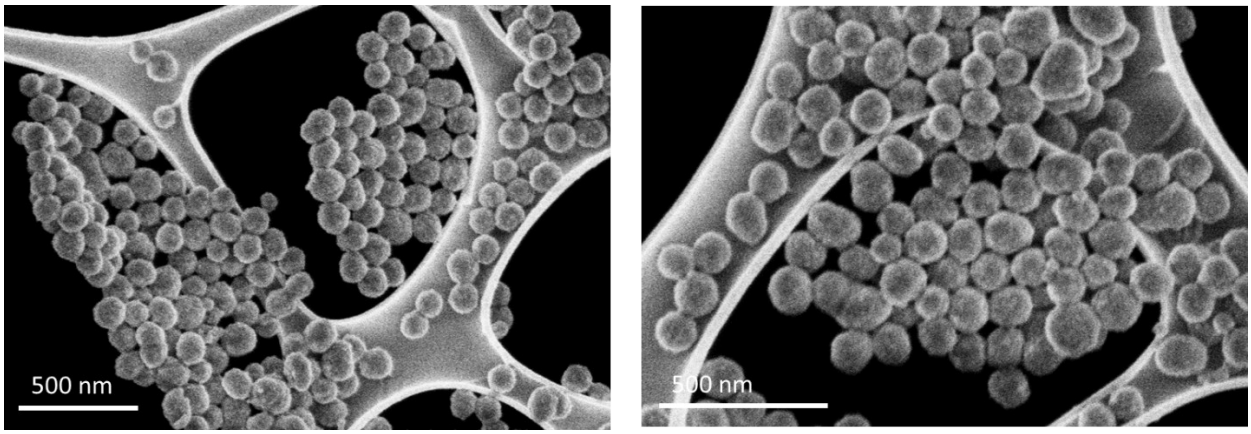


Figure S4: STEM images of **PDA**

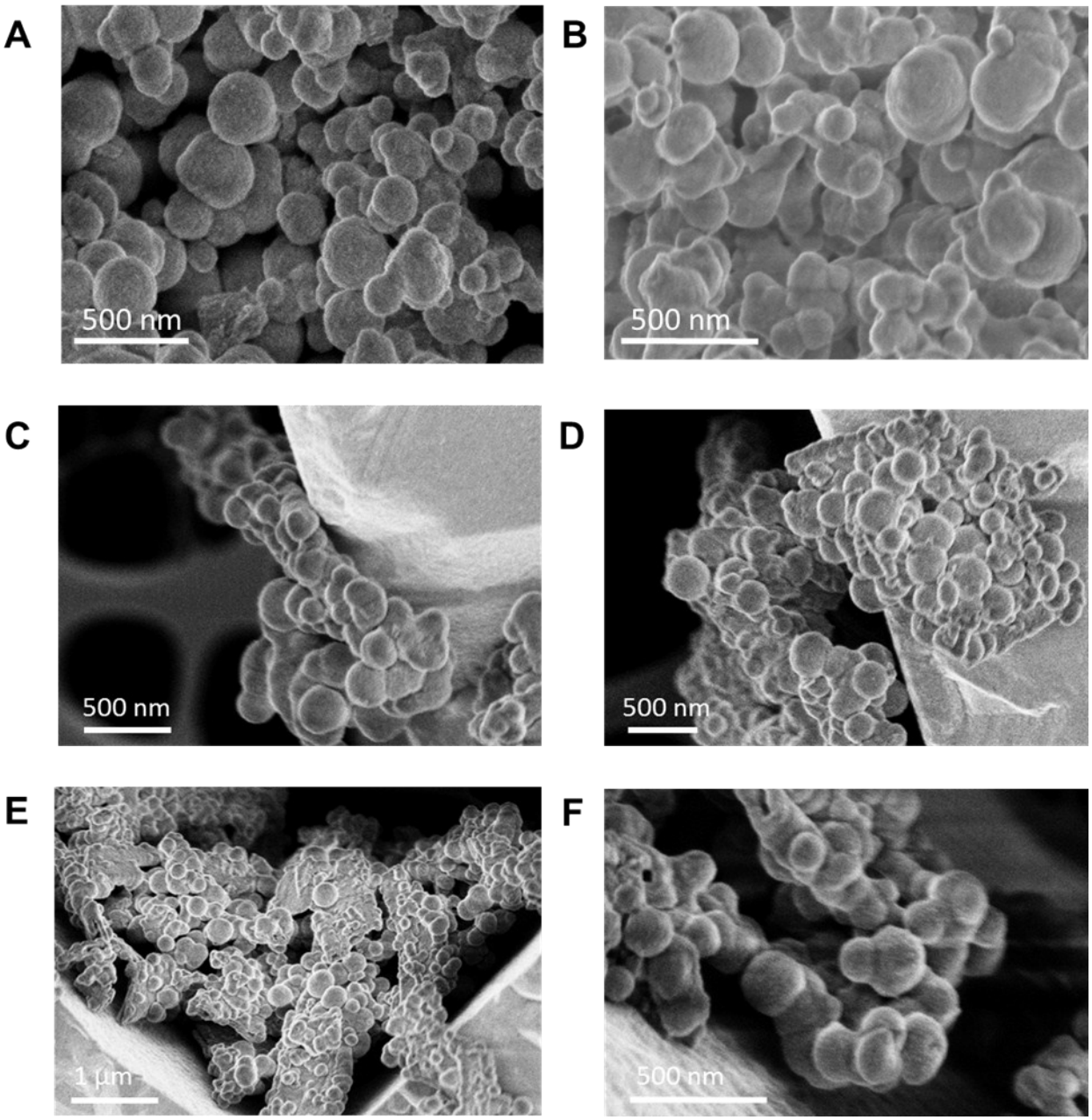


Figure S5: STEM images of **FLPDA-5** before (A and B), post photooxidation reaction (C and D) and post photoreduction reaction (E and F) showing no considerable changes to size or shape of the particles.

2.3 UV-Vis Absorption Spectra

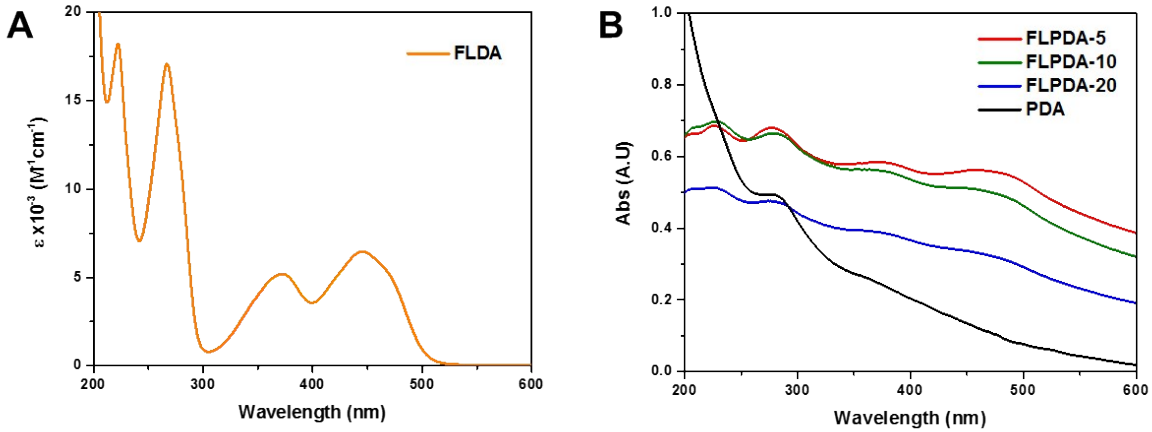


Figure S6: (A) **FLDA** (80 µM) in water (B) **FLPDA** samples (50 µg/mL) and PDA (25 µg/mL) in 0.05 M KPi buffer pH 7.4.

2.4 Fluorescence Emission spectra

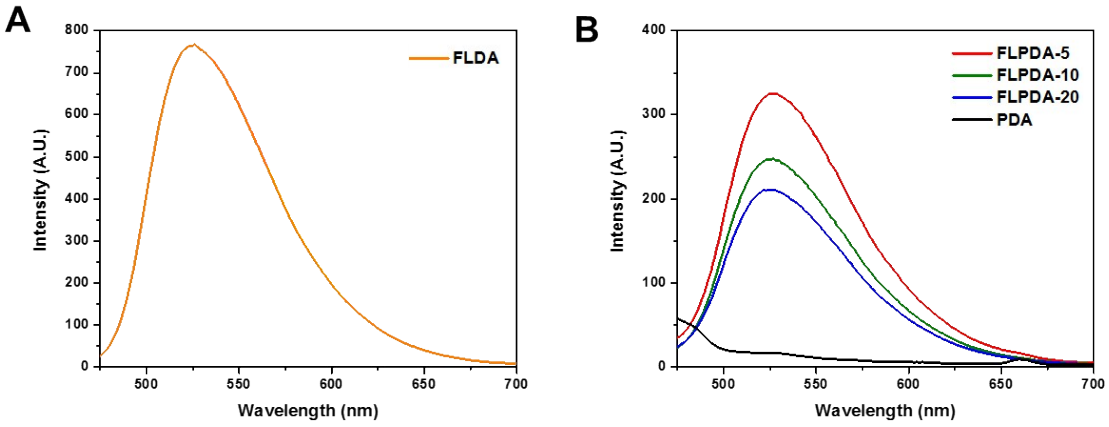


Figure S7: (A) **FLDA** (80 µM) in water (B) **FLPDA** samples and PDA – 50 µg/mL in 0.05 M KPi buffer pH 7.4

2.5 DOPAC-Free Flavin (FLOH) Comparative Analysis

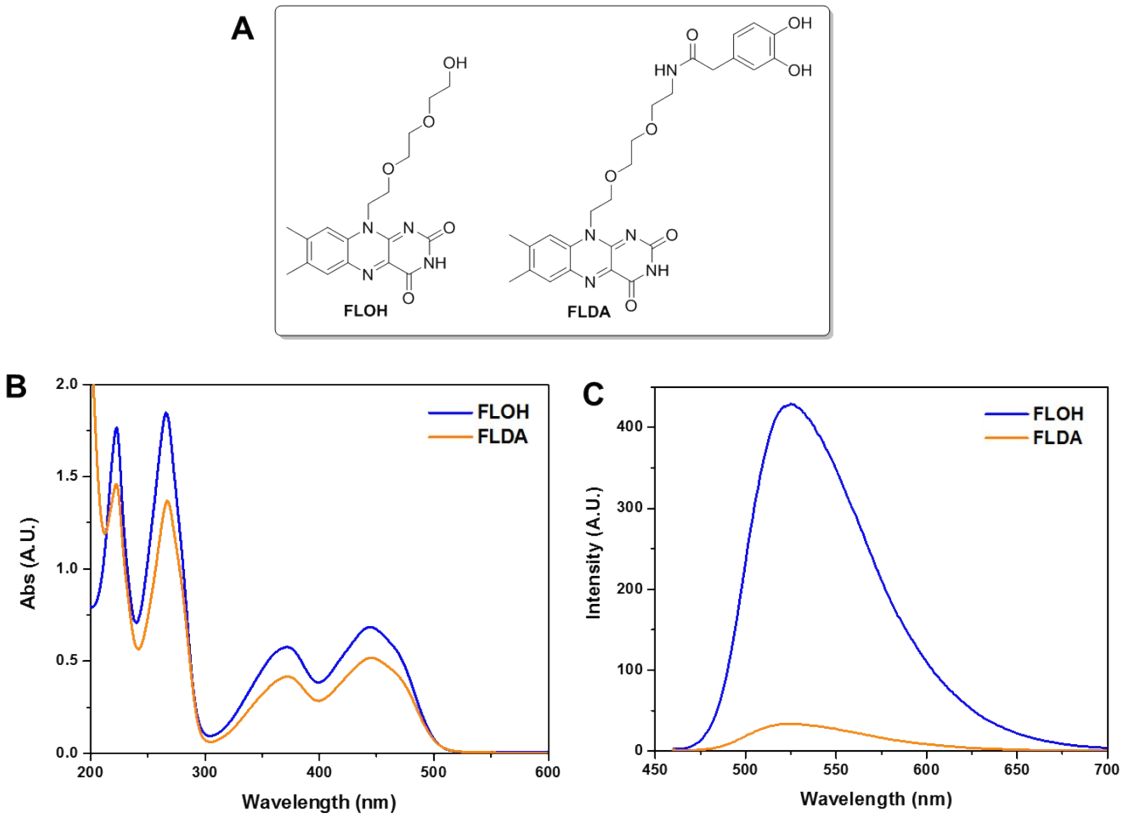


Figure S8: (A) Structures of DOPAC-free flavin, **FLOH** and **FLDA**. (B) UV-Vis spectra of compounds – both 100 µM in water. (C) Fluorescence spectra of compounds – both 20 µM in water (ex/em slits = 5 nm).

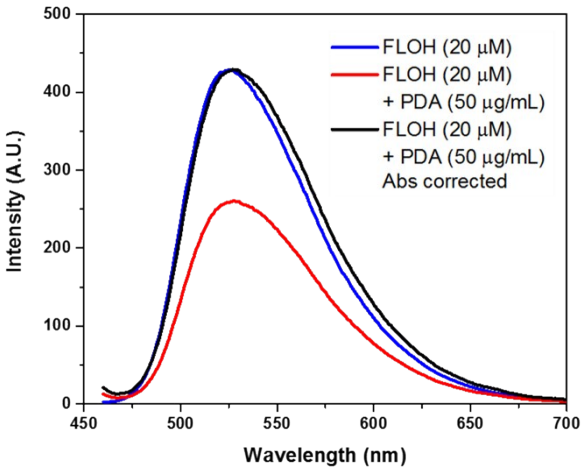


Figure S9: Fluorescence spectra of **FLOH** (20 µM), **FLOH** (20 µM) incubated with PDA (50 µg/mL) and the absorbance corrected spectrum of **FLOH** (20 µM) incubated with PDA (50 µg/mL). The latter was calculated by multiplying the λ_{max} intensity value at 527 nm by the percentage absorbance of PDA (50 µg/mL) at λ = 450 nm (41%) followed by the percentage absorbance of PDA at λ = 527 nm (17%). This results in a representative emission spectrum of **FLOH** showing that quenching by PDA is negligible and that any attenuation in intensity is absorbance dependant.[5]

2.6 Fluorescence Calibration Curve

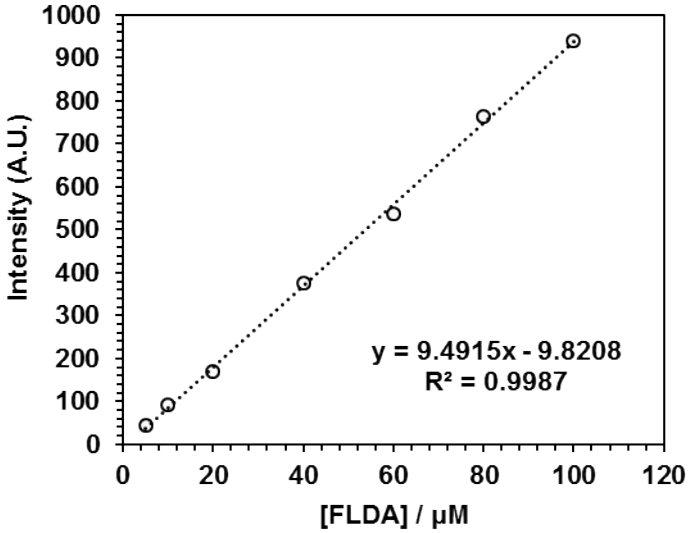


Figure S10: Fluorescence calibration curve of **FLDA** at λ_{527} in 0.05 M KPi buffer pH 7.4 (ex/em slits = 10 nm). All measurements were carried out in 200 µL total volume.

Table S2: Correlation data obtained from the calibration curve. All measurements were carried out in 0.05 M KPi buffer pH 7.4 and 200 µL total volume (ex/em slits = 10 nm).

Sample	**Monomer molar ratio** (DA:FLDA)	**Intensity @ λ_{527}** (50 µg/mL)	**Absorbance Corrected Intensity** (50 µg/mL PDA)	**Relative [FLDA]** (µM)	**Approximate FLDA content** (µmol/mg FLPDA)
FLPDA-5	5:1	325.35	536.73	55.51	1.11
FLPDA-10	10:1	248.16	409.39	42.10	0.84
FLPDA-20	20:1	210.82	347.79	35.61	0.71

2.7 FTIR

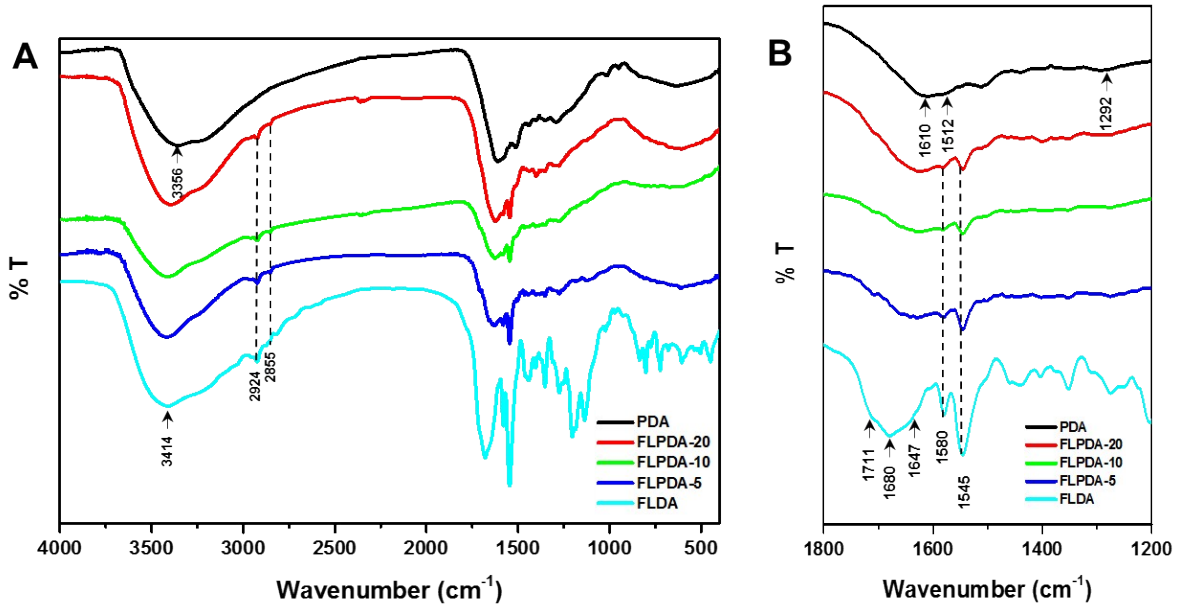


Figure S11: (A) Full scale FTIR spectra of **PDA**, **FLPDA-20**, **FLPDA-10**, **FLPDA-5** and **FLDA**. (B) Zoomed scale FTIR spectra of **PDA**, **FLPDA-20**, **FLPDA-10**, **FLPDA-5** and **FLDA**.

The spectrum of PDA (Figure S10A) contains characteristic bands at 3356 cm^{-1} relating to O-H and N-H stretching vibrations. The spectrum of **FLDA** has a much broader band at 3414 cm^{-1} relating to these vibrations, and as a result, **FLPDA** samples show very similar bands through the contributions of both modes. Unlike PDA, the spectra of **FLPDA** samples clearly contain C-H stretching vibration bands at 2924 and 2855 cm^{-1} that correlate well to the spectrum of **FLDA**. As seen in Figure S10B, the main characteristic bonds corresponding to the flavin moieties in the polymer can clearly be observed when compared to the monomer **FLDA**, from the sharp bands at 1545 cm^{-1} and 1580 cm^{-1} relating to ν(C=N) modes in the isoalloxazine ring.[6] Contributions from the flavin carbonyl ν(C=O) (1711 and 1680 cm^{-1}) and ν(C=C) be seen in **FLPDA** samples in combination with C=O and C-O vibrational modes of PDA, seen at 1610 and 1512 cm^{-1} respectively in its spectrum. Further C=N and C=C combined contributions can also be observed at lower wavenumbers in the spectra of **FLPDA** (1292 cm^{-1}).

2.8 Amplex Red Photooxidation

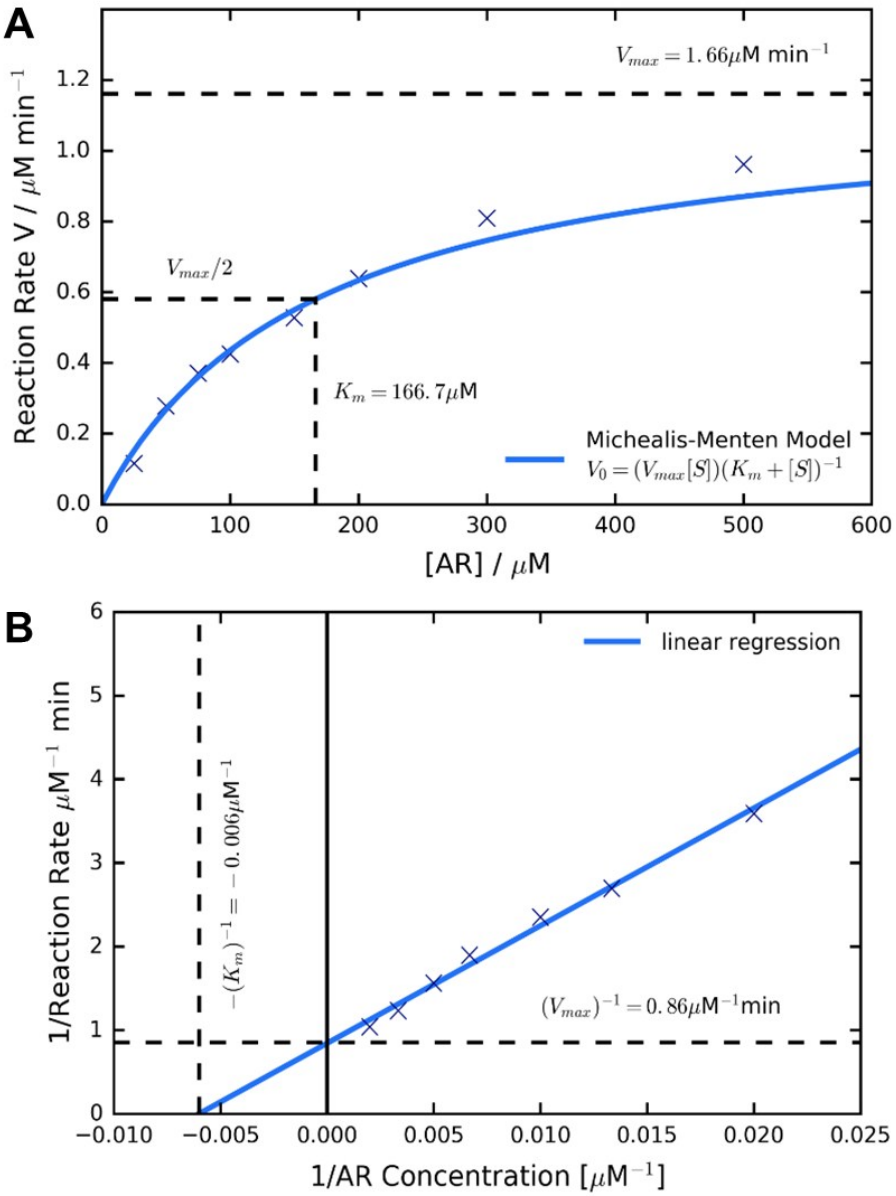


Figure S12: Michaelis-Menten plot (A) and reciprocal Lineweaver-Burke plot (B) for the photooxidation of **AR** by **FLPDA-5** (10 µg/mL) in KPi buffer (10 mM, pH 7.4) using 50.3 mW laser diode (458 nm).

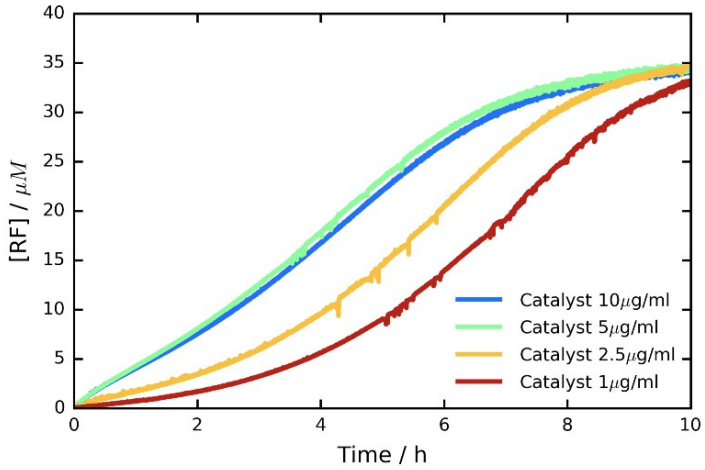


Figure S13: Plot of **FLPDA-5** concentration dependence on the photooxidation of **AR** (100 µM) in KPi buffer (10 mM, pH 7.4) using 9.32 mW laser diode (458 nm).

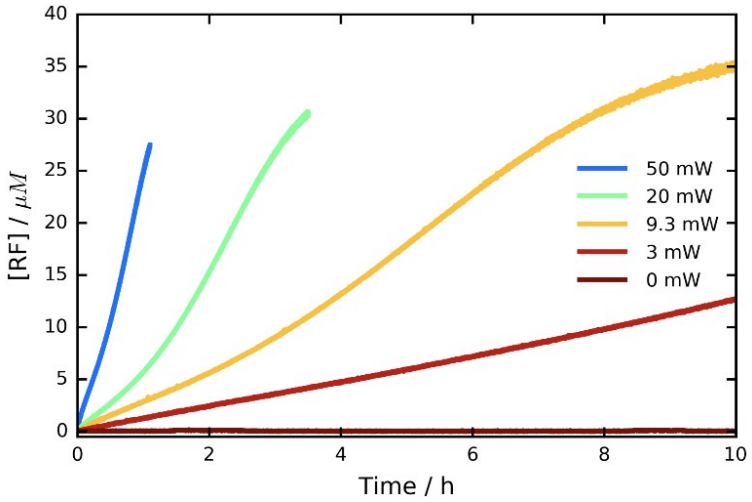


Figure S14: Plot of laser diode power (458 nm) dependence (including dark measurement at 0 mW) on the photooxidation of **AR** (100 µM) in KPi buffer (10 mM, pH 7.4).

2.9 Resazurin Photoreduction Recycles

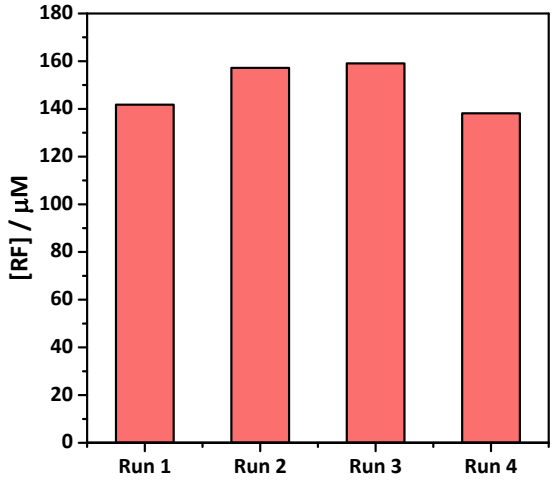


Figure S15: Recycling experiments were carried out by irradiating **FLPDA-5** (50 µg/mL) in the presence of **RZ** (0.2 mM) and EDTA (1 mM) in KPi buffer (0.1 M, pH 7.4) for 1 hour under Ar atmosphere. The catalyst was then removed by centrifugation (4000 rpm, 30 min) and washed with water (2 x 10 mL).

2.10 Photostability Investigations

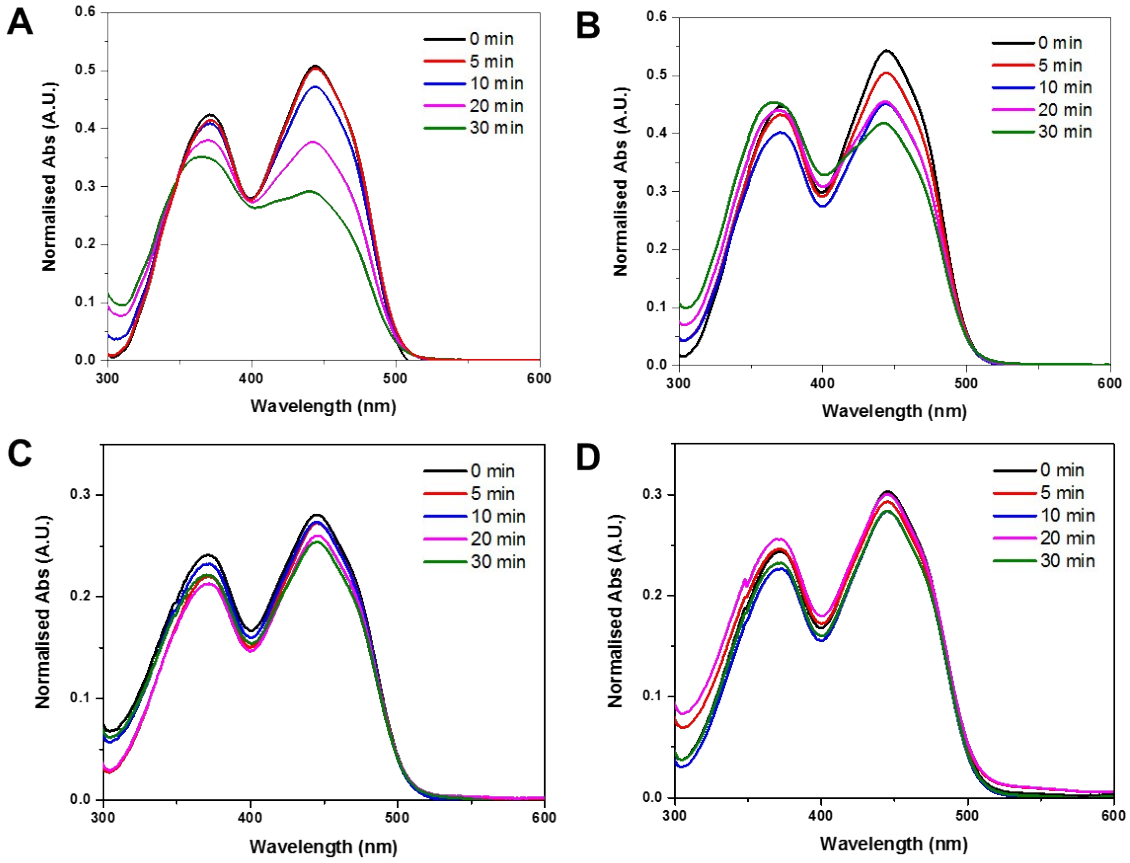


Figure S16: Changes in UV-Vis absorbance spectra over time for homogenous flavin compounds (0.5 mM, 50 mM KPi buffer pH 7.4) during irradiation (50 mW, 458 nm) in air (A - **FLOH**, C – **FLDA**) and under N_2 atmosphere (B – **FLOH**, D - **FLDA**). All spectra are 10x dilutions from the orginal mixtures.

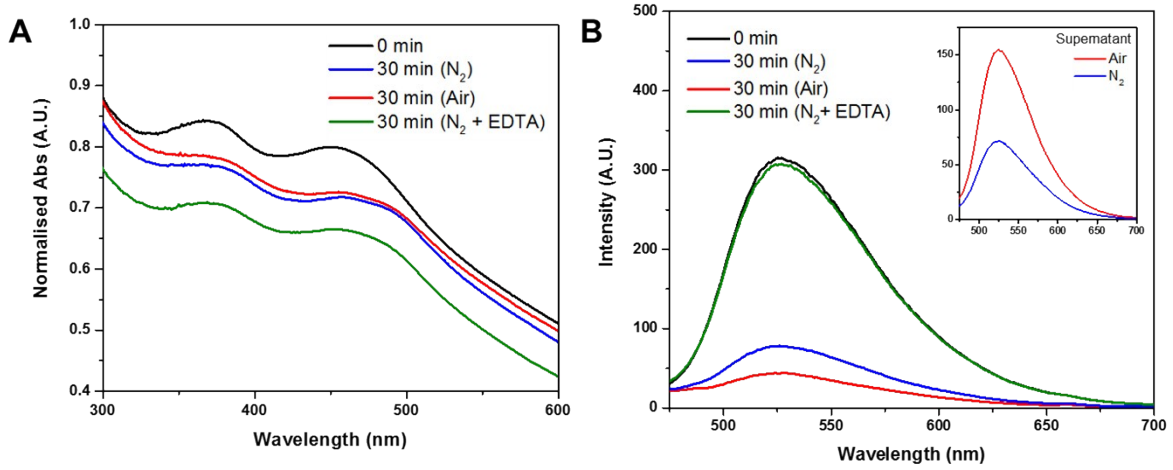
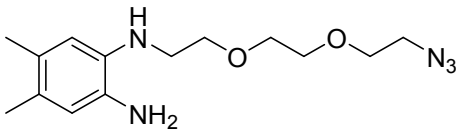


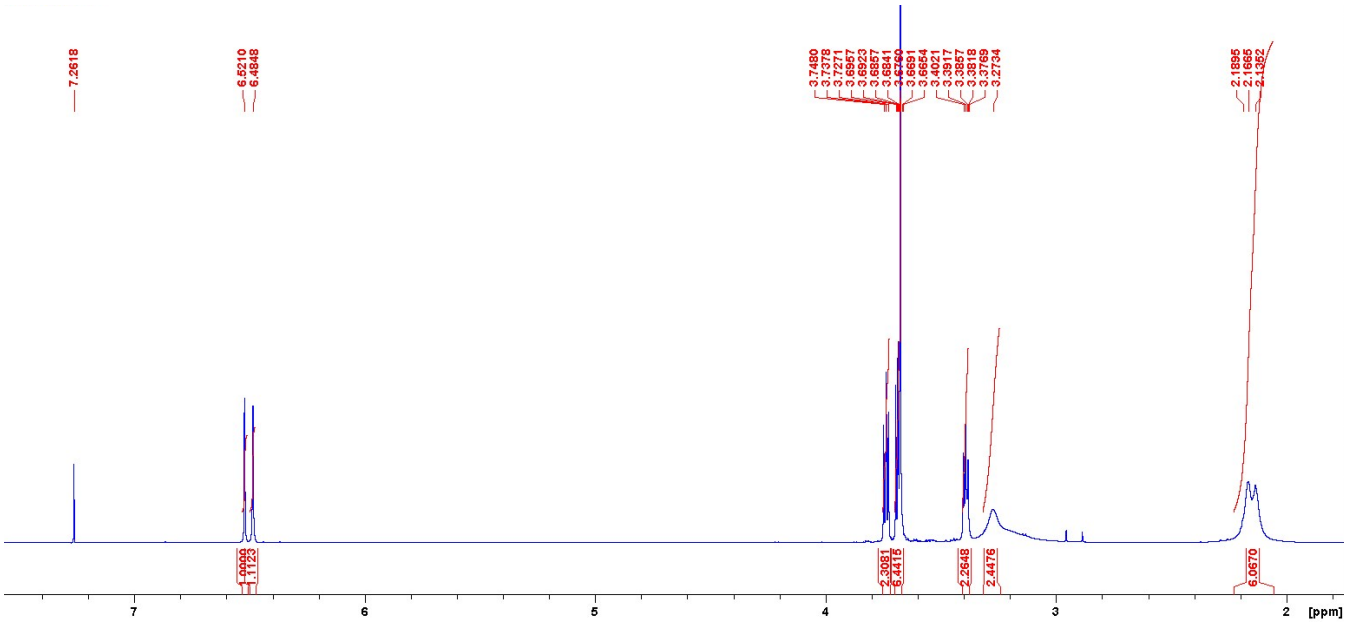
Figure S17: Changes in UV-Vis absorbance spectra for **FLPDA-5** (50 µg/mL, 50 mM KPi buffer pH 7.4) before and after 30 min irradiation (50 mW, 458 nm) in air (A) and under N_2 atmosphere (B). Fluorescence spectra of **FLPDA-5** after irradiation in air and under N_2 atmosphere (C) and respective supernatants after centrifugation of particles (inset)**.**

3 ^{1}H and ^{13}C NMR Spectra

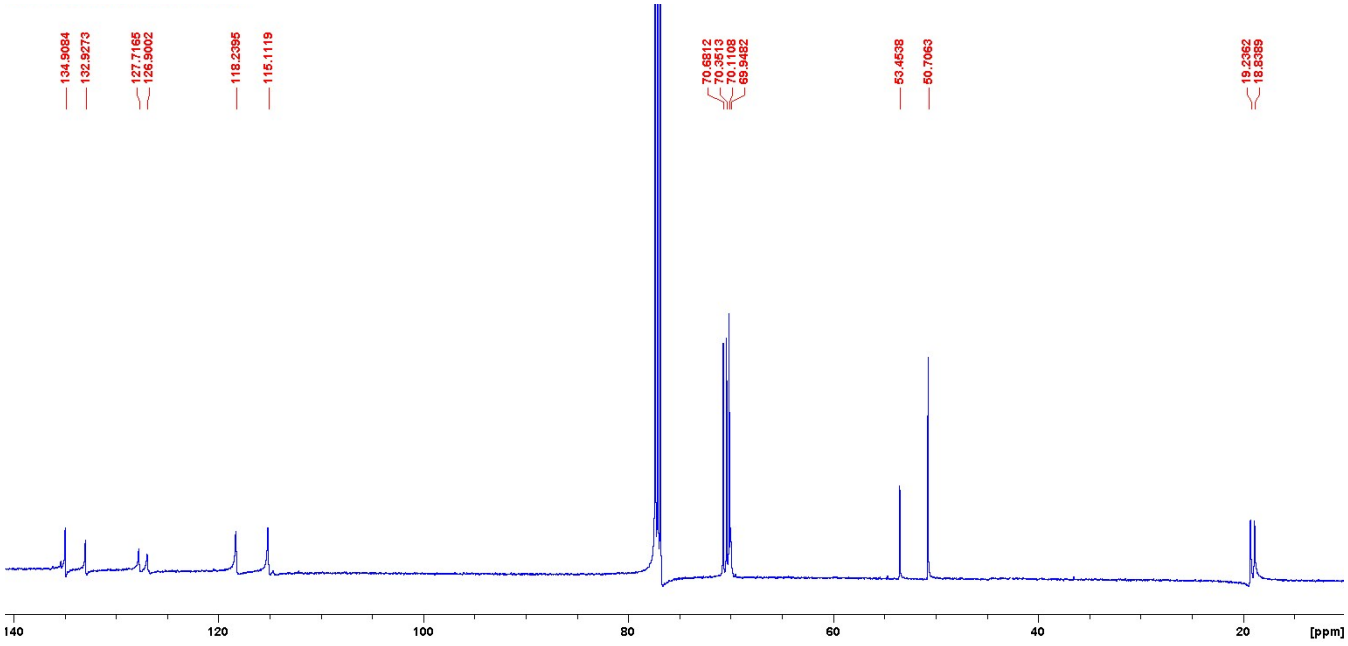
***N*^{1}-(2-(2-(2-azidoethoxy)ethoxy)ethyl)-4,5-dimethylbenzene-1,2-diamine (2):**



^{1}H NMR (500 MHz, $CDCl_3$):

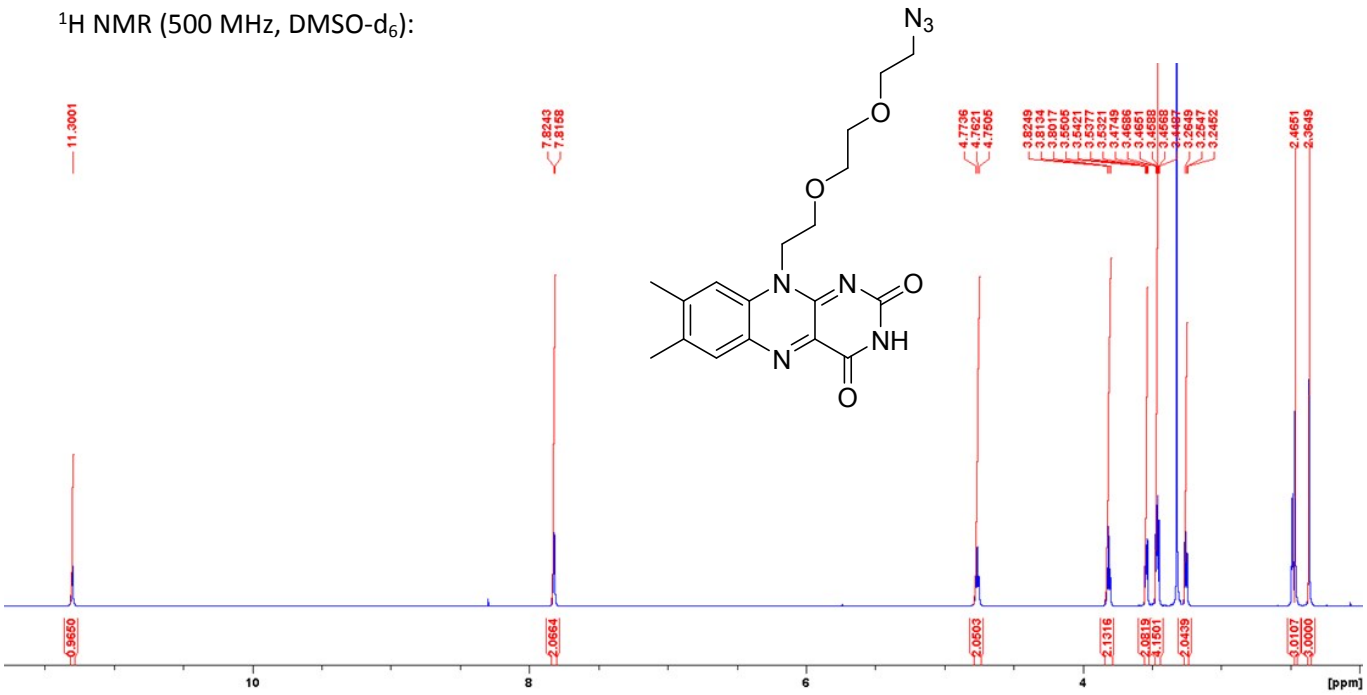


^{13}C NMR (500 MHz, CDCl3):

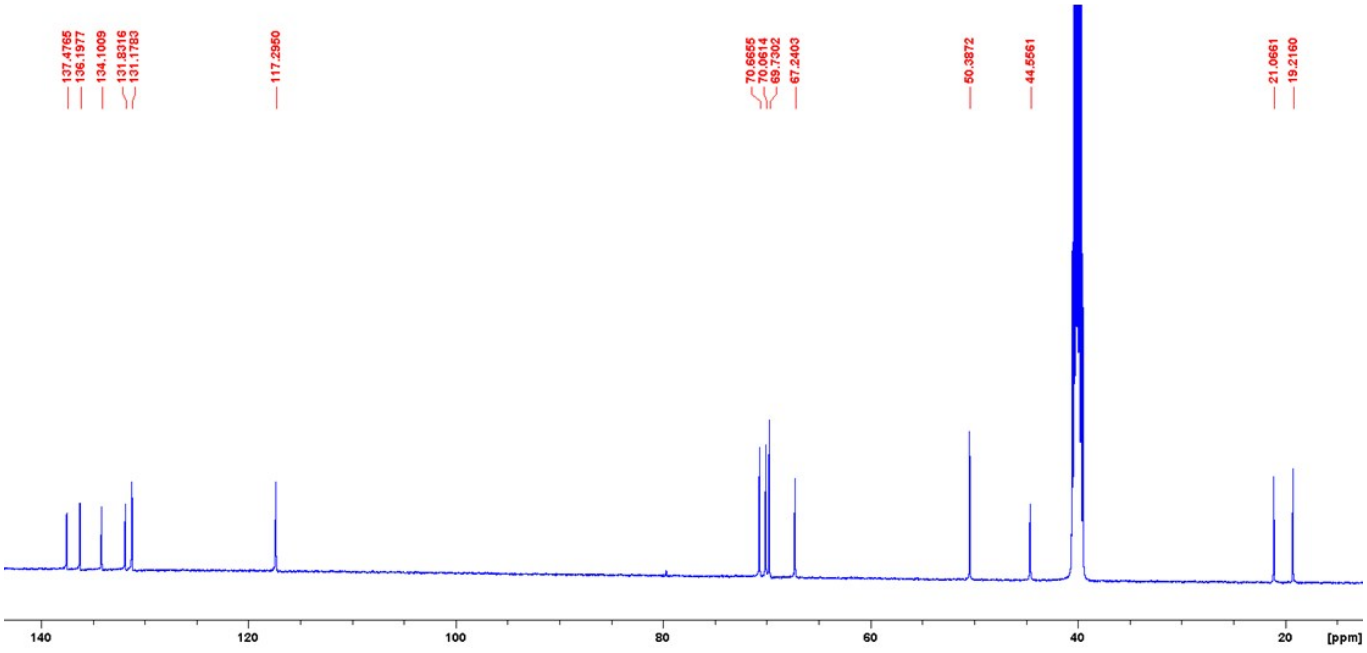


10-(2-(2-(2-Azidoethoxy)ethoxy)ethyl)-7,8-dimethylbenzo[g]pteridine-2,4(3H,10H)-dione (3):

^{1}H NMR (500 MHz, DMSO-d_6):

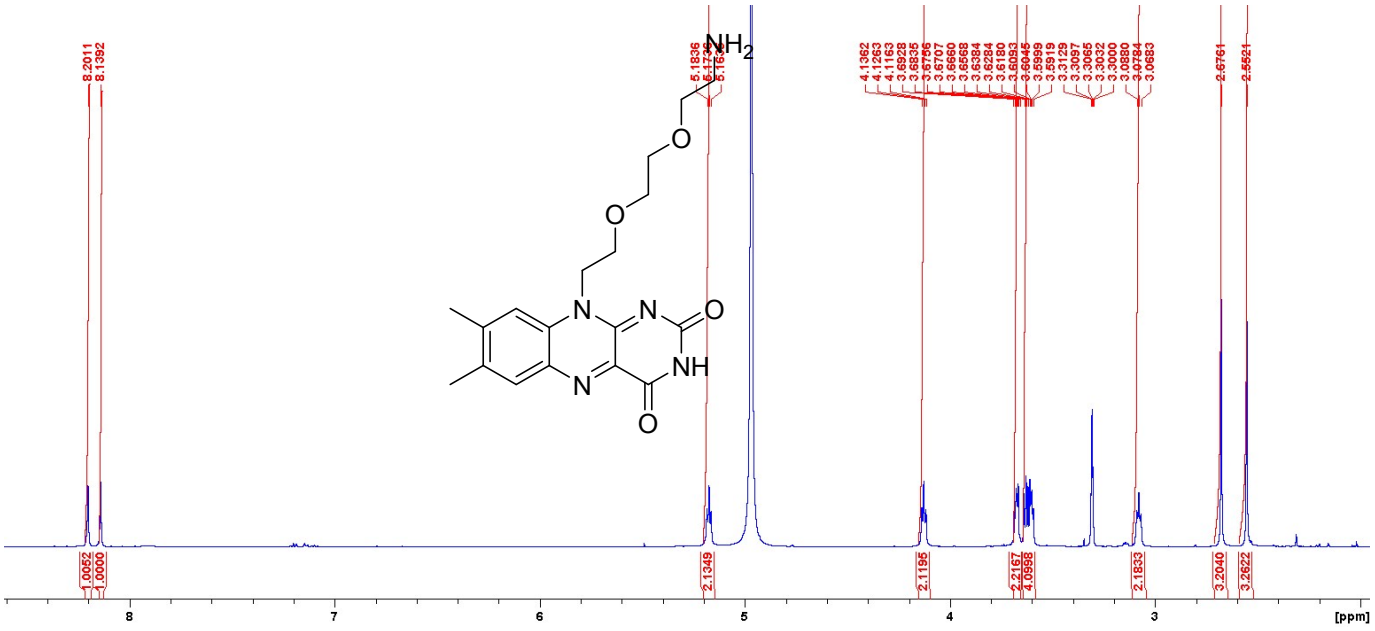


^{13}C NMR (500 MHz, DMSO-d_6):

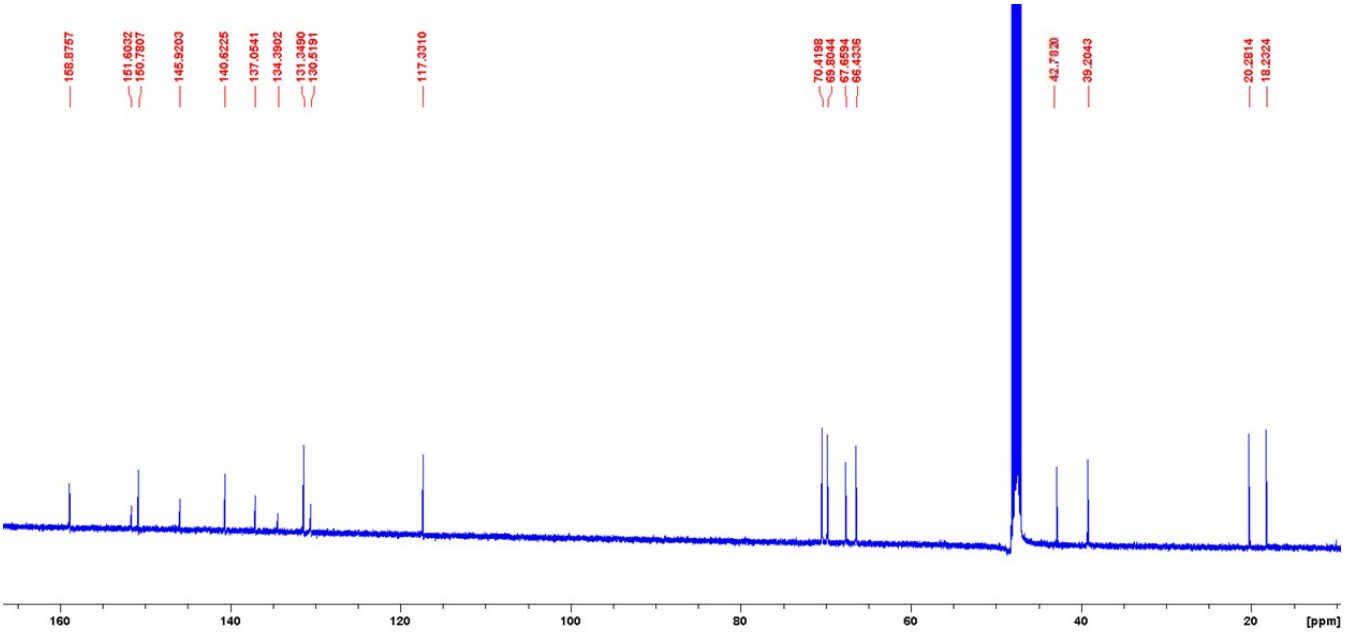


10-(2-(2-(2-aminoethoxy)ethoxy)ethyl)-7,8-dimethylbenzo[g]pteridine-2,4(3H,10H)-dione (4):

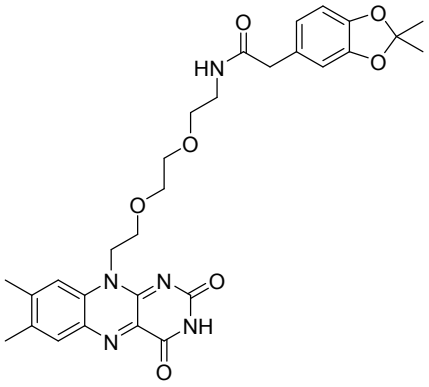
^{1}H NMR (500 MHz, CD_3OD):



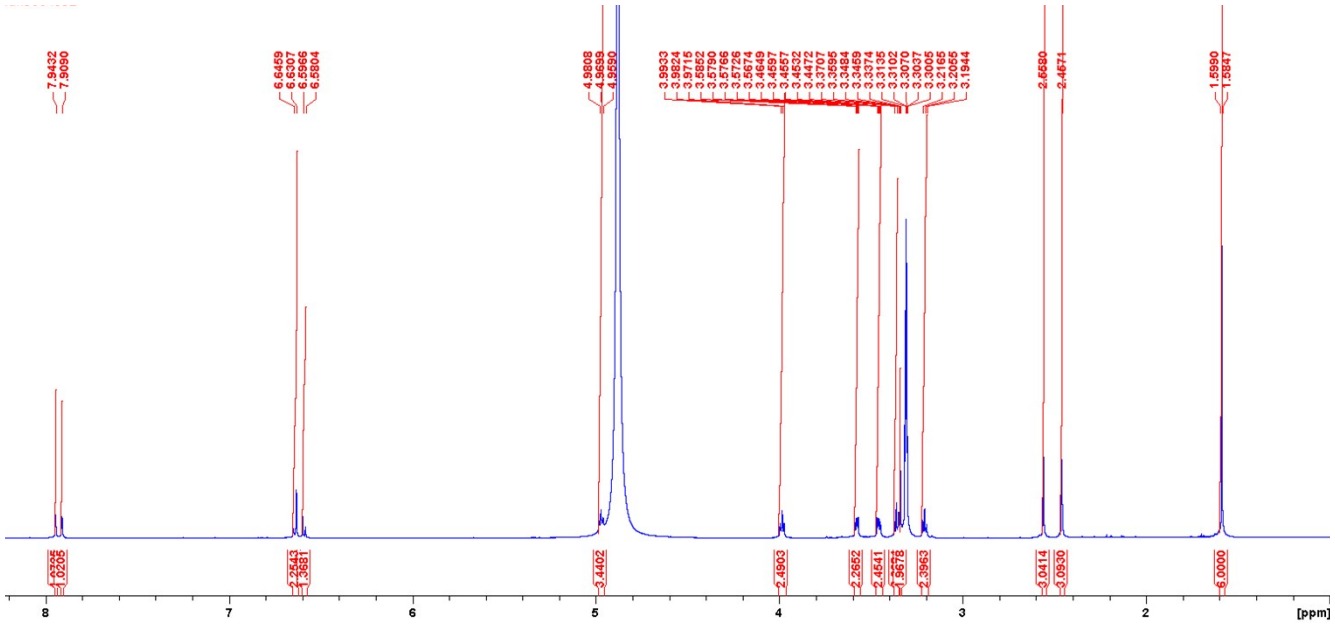
^{13}C NMR (500 MHz, CD_3OD):



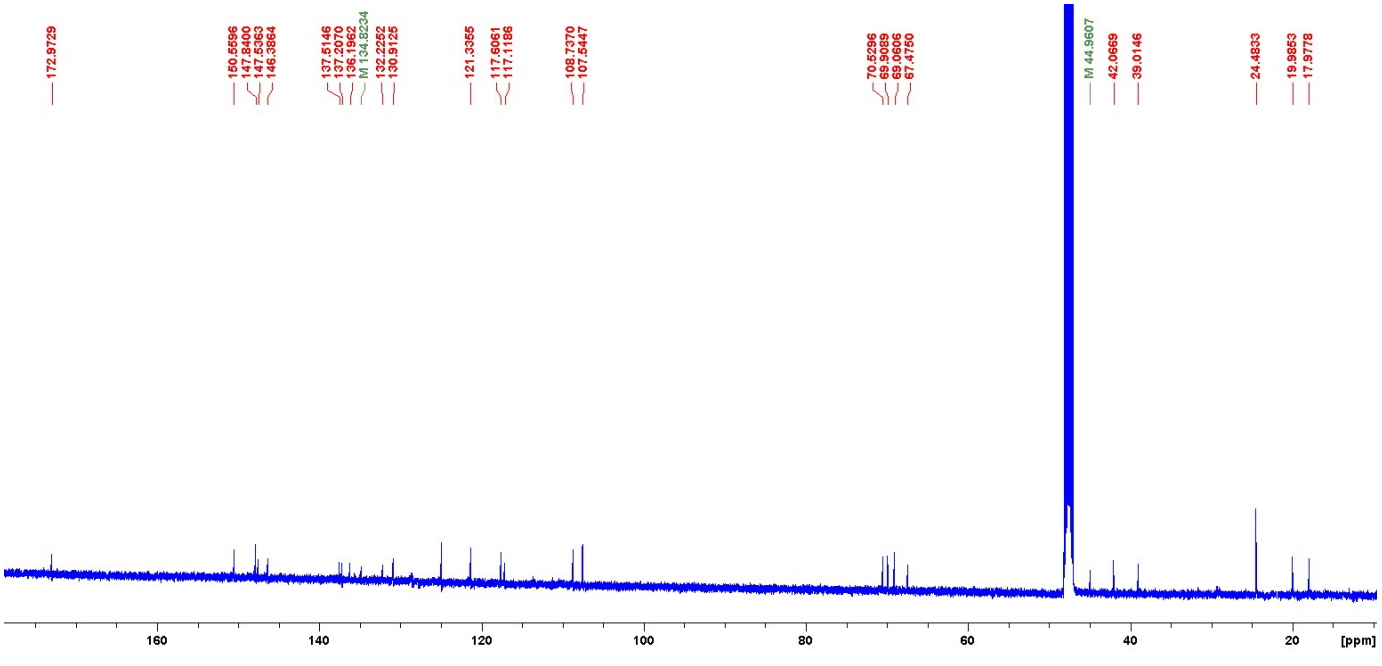
N-(2-(2-(2-(7,8-Dimethyl-2,4-dioxo-3,4-dihydrobenzo[g]pteridin-10(2H)-yl)ethoxy)ethoxy)ethyl)-2-(2,2-dimethylbenzo[d][1,3]dioxol-5-yl)acetamide (6):



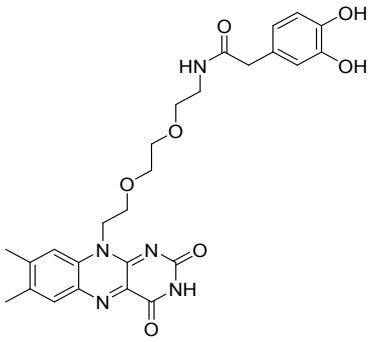
^{1}H NMR (500 MHz, CD_3OD):



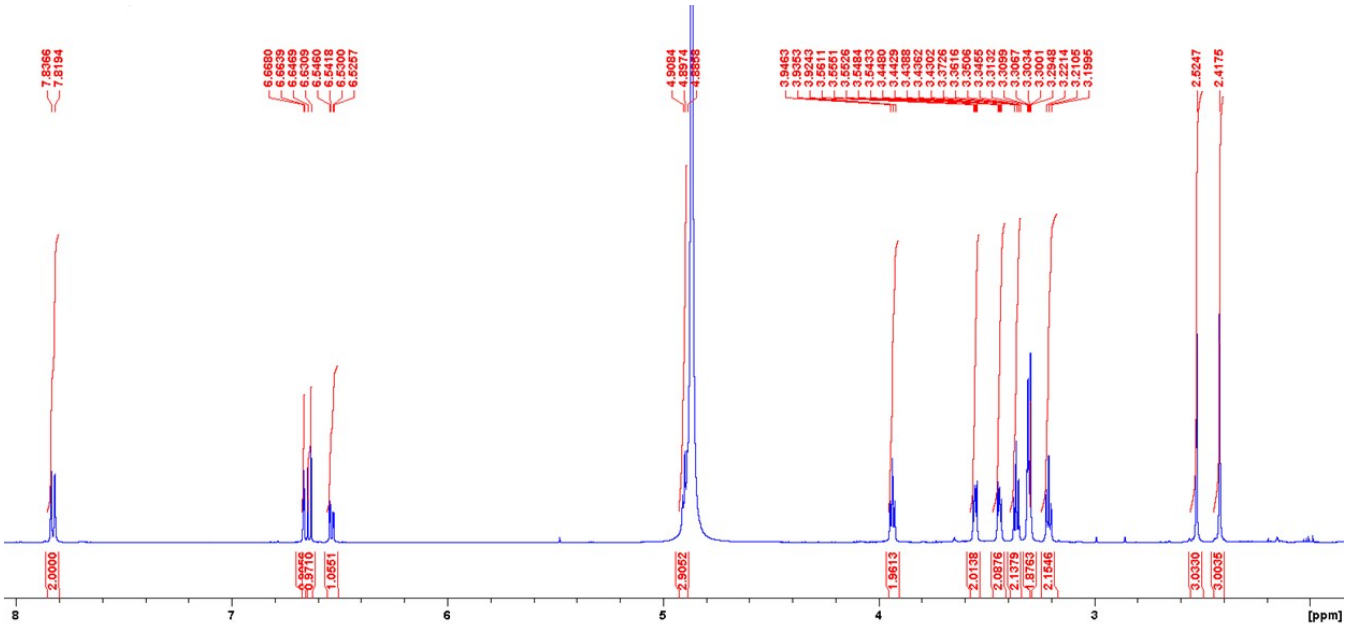
^{13}C NMR (500 MHz, CD_3OD):



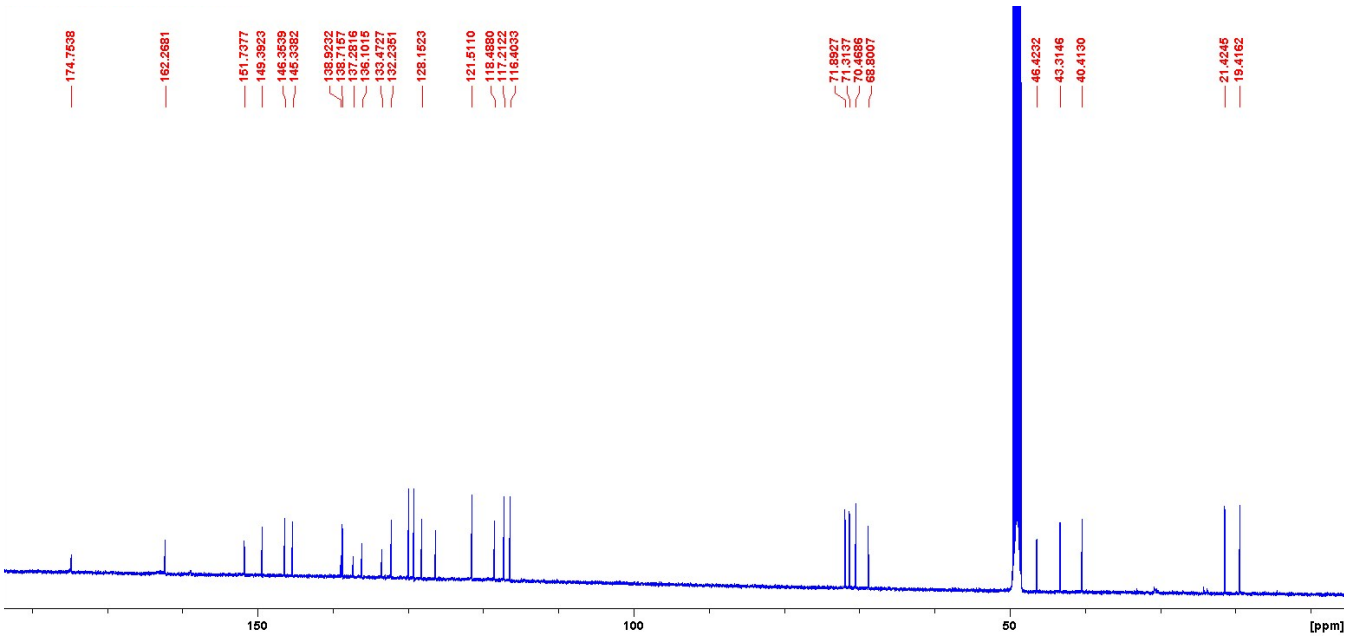
2-(3,4-Dihydroxyphenyl)-N-(2-(2-(2-(7,8-dimethyl-2,4-dioxo-3,4-dihydrobenzo[g]pteridin-10(2H)-yl)ethoxy)ethoxy)ethyl)acetamide (FLDA):



^{1}H NMR (500 MHz, CD_3OD):

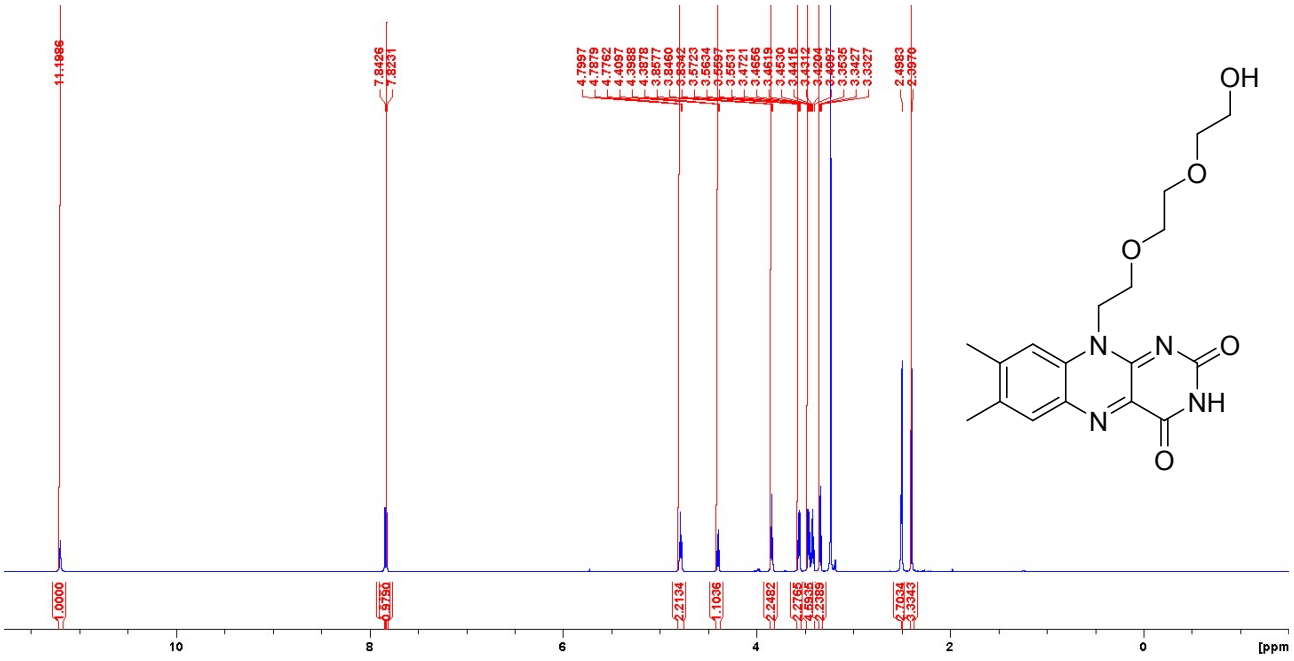


^{13}C NMR (500 MHz, CD_3OD):

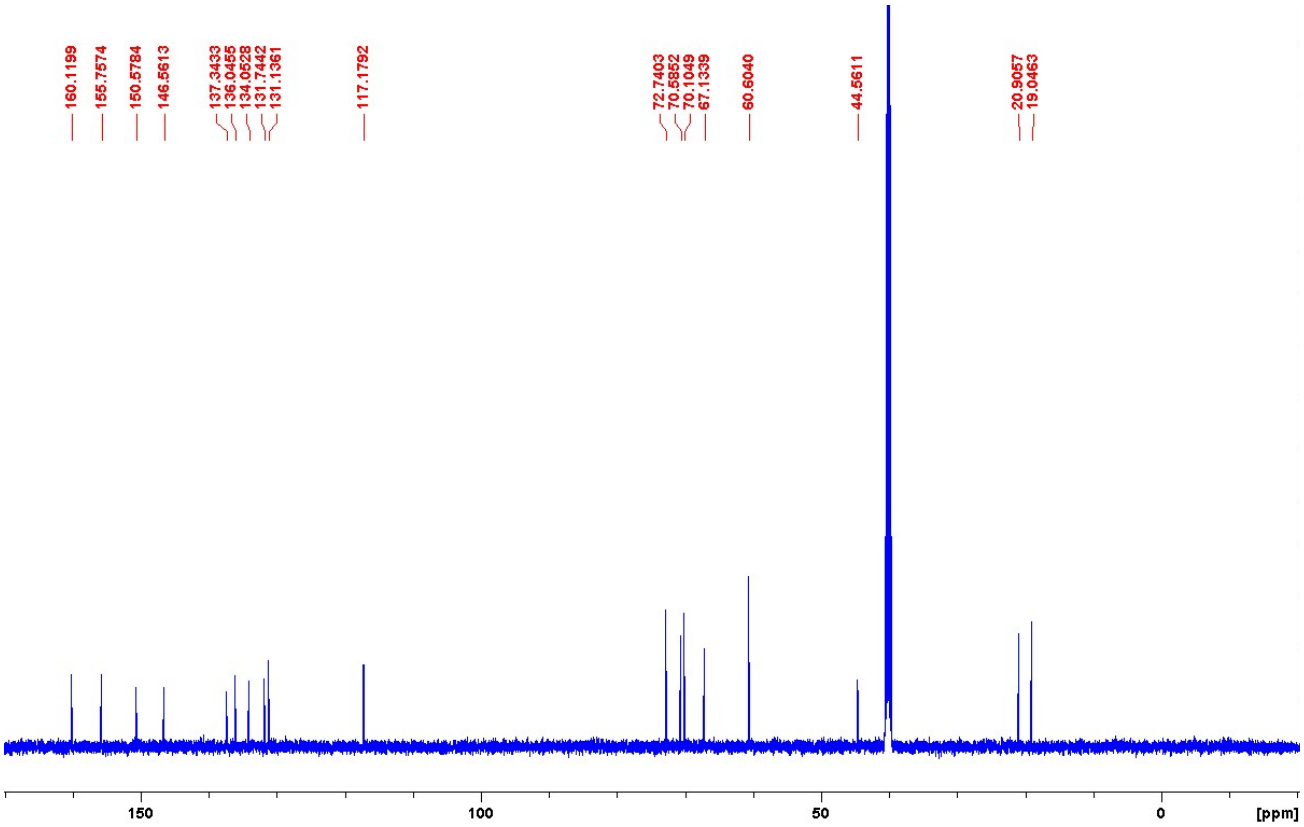


10-(2-(2-(2-hydroxyethoxy)ethoxy)ethyl)-7,8-dimethylbenzo[g]pteridine-2,4(3H,10H)-dione (FLOH):

^{1}H NMR (500 MHz, DMSO-d_6):



^{13}C NMR (500 MHz, DMSO-d_6):



4 References

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