# Electronic Supplementary Information

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## 2 Light Source

The light source used for experiments was an EvoluChem 6200K white LED (18W, 400-700 nm, $\lambda_{max}$ = 445 nm, Hepatochem, US) with emission spectrum as shown in **Figure S1**. Illuminance was measured using an RS PRO IM720 Light Meter. Irradiance of emission was measured using a Thor Labs PM100D with a S120VC sensor.

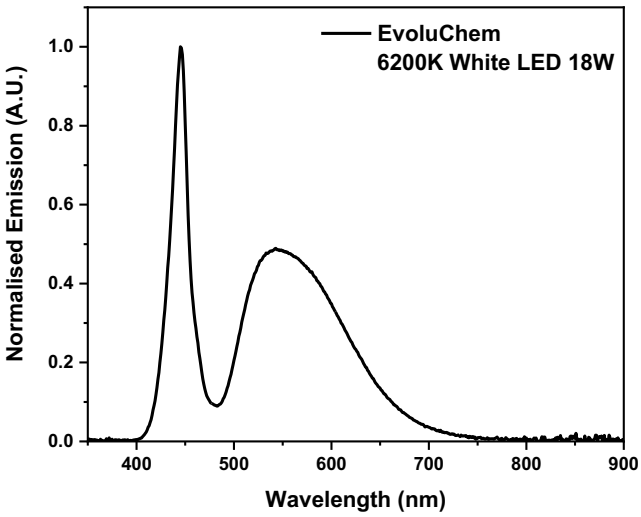



**Figure S1:** Emission spectrum of EvoluChem 6200K white LED light source.

# 3 Synthesis and Characterisation of Flavins

## *3.1 Materials and Methods*

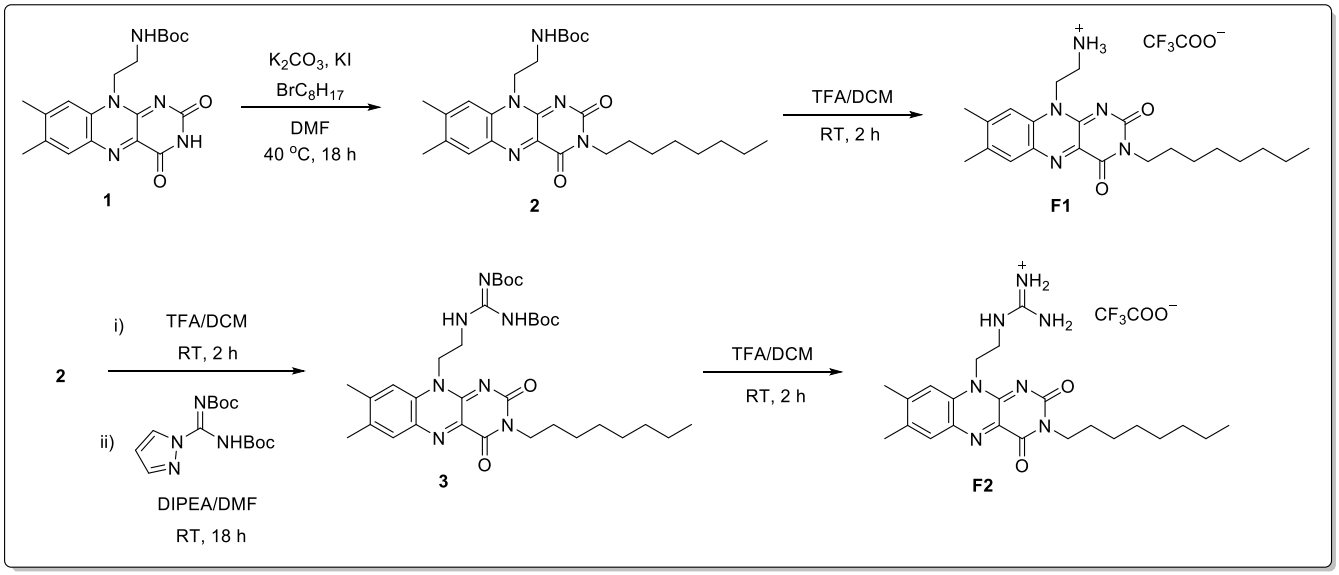
All materials were purchased from either Fisher Scientific (UK), Acros Organics (UK), Alfa Aeser (UK), Sigma-Aldrich (UK) or TCI Chemicals (BE) in the highest purity available and used without further purification. For column chromatography 40-63 µm silica gel (Merck) was used as stationary phase. Analytical thin layer chromatography (TLC) was performed on aluminum foil pre-coated with $SiO_2$-60 F254 (Merck) and visualized with a UV-lamp (254 and 365 nm).

## *3.2 Characterisation techniques*

$^{1}H$ and $^{13}C$ NMR measurements were carried out using a 400 MHz QNP Cryoprobe Spectrometer (Bruker). The resonance multiplicity is abbreviated as: s (singlet), d (doublet), t (triplet), q (quadruplet), quint (quintet), sext (sextet), sep (septet), m (multiplet) and br (broad). The chemical shift δ is expressed in "ppm" and the coupling constant J is in "Hz". The chemical shifts are referenced to the residual solvent peak as the internal standard. 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 slits of 5 nm.

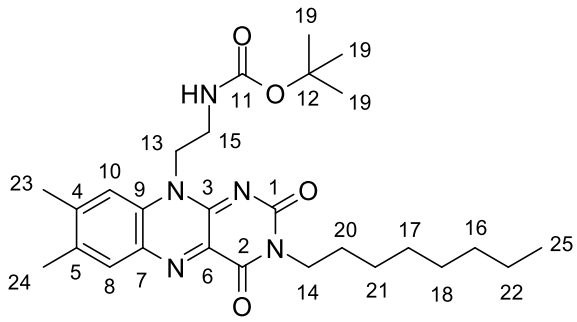
### *3.3 Flavins F1 and F2*

Flavin **1** was prepared according to the protocol from ref. 1




**Scheme S1:** Synthesis of dimethyl flavins **F1** and **F2.**

***tert*-Butyl (2-(7,8-dimethyl-3-octyl-2,4-dioxo-3,4-dihydrobenzo[*g*]pteridin-10(2*H*)-yl)ethyl)carbamate (2):**

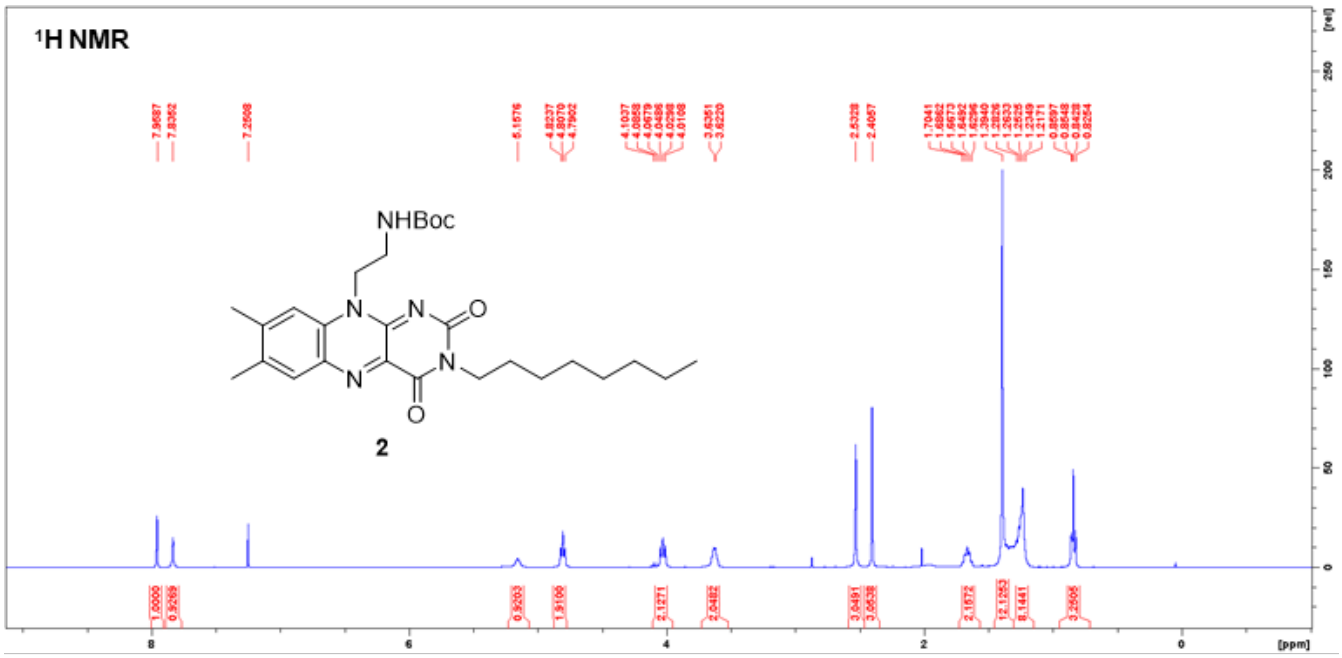


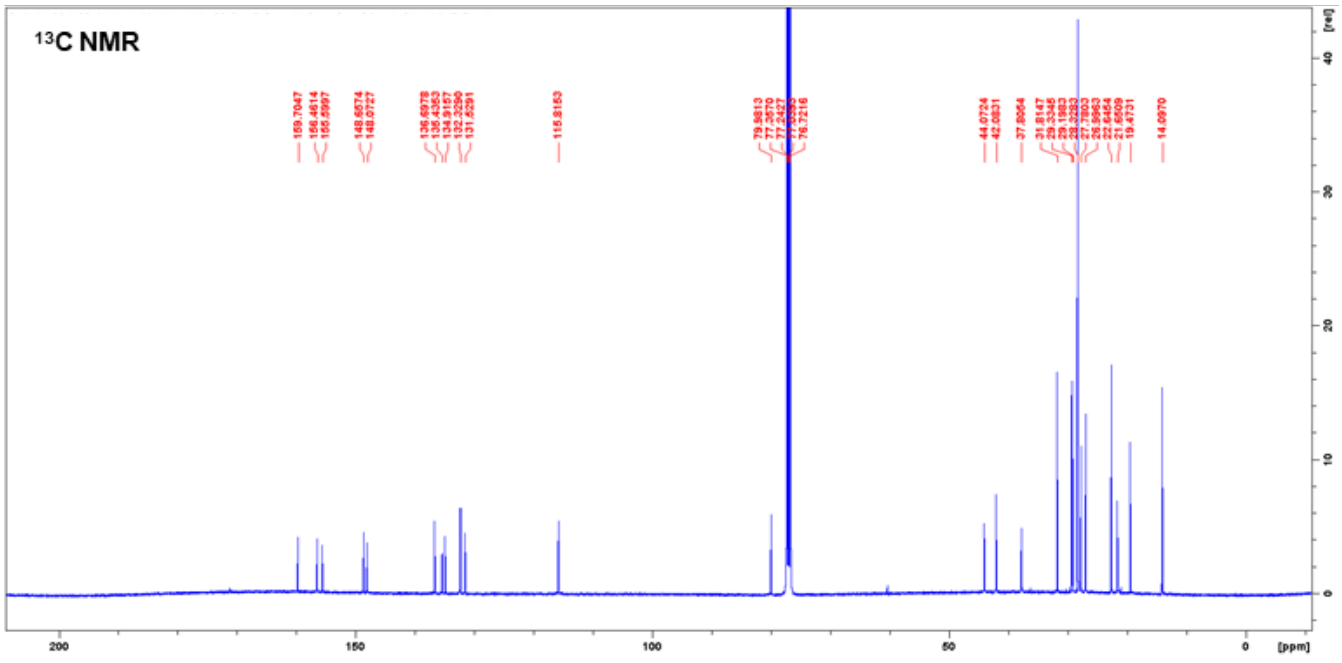
Flavin **1** (0.50 g, 1.30 mmol, 1.0 equiv.)>, anhydrous $K_2CO_3$ (0.27 g, 1.95 mmol, 1.5 equiv.) and anhydrous KI (21.5 mg, 0.130 mmol, 0.1 equiv.) were suspended in anhydrous DMF (50 mL) under Ar atmosphere. The mixture was then heated to 40 °C and 1-bromooctane (1.26 g, 1.12 mL, 6.50 mmol, 5.0 equiv.) dissolved in anhydrous DMF (5 mL) was added dropwise. The mixture was then stirred for 18 h at 40 °C under Ar atmosphere in the dark. The solvent was then removed under reduced pressure and the resulting residue was suspended in EtOAc (20 mL) and washed with water (3 x 20 mL) and brine (20 mL). The organic phase was then dried over $Na_2SO_4$, filtered, and concentrated under reduced pressure. The resulting orange residue was purified by silica gel column chromatography (1:2 EtOAc/DCM to 1:1 EtOAc/DCM) to yield flavin **2** as an orange solid (0.544 g, 70%).

**$^1$H NMR** (400 MHz, $CDCl_3$) δ = 7.96 (s, 1H, **H8**), 7.84 (s, 1H, **H10**), 5.16 (br t, 1H, -N**H**-), 4.81 (t, *J* = 6.7 Hz, 2H, **H14**), 4.03 (t, *J* = 7.6 Hz, 2H, **H13**), 3.62 (br m, 2H, **H15**), 2.53 (s, 3H, **H23**), 2.41 (s, 3H, **H24**), 1.67 (quint, *J* = 7.4 Hz, 2H, **H20**), 1.39 (s, 9H, **H19**), 1.36-1.22 (m, 10H, **H16**/**17**/**18**/**21**/**22**), 0.84 (t, *J* = 6.9 Hz, 3H, **H25**) ppm.

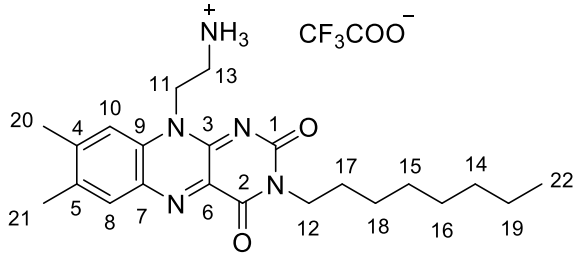
**$^{13}$C NMR** (100 MHz, $CDCl_3$) δ = 159.7 (**C1**), 156.5 (**C11**), 155.6 (**C2**), 148.7 (**C3**), 148.1 (**C4**), 136.7 (**C5**), 135.4 (**C6**), 134.9 (**C7**), 132.3 (**C8**), 131.5 (**C9**), 115.8 (**C10**), 80.0 (**C12**), 44.1 (**C13**), 42.1 (**C14**), 37.8 (**C15**), 31.8 (**C16**), 29.3 (**C17**), 29.2 (**C18**), 28.3 (**C19**), 27.8 (**C20**), 27.0 (**C21**), 22.6 (**C22**), 21.7 (**C23**), 19.5 (**C24**), 14.1 (**C25**) ppm.

**HRMS (ESI)** m/z: $[M + H]^+$ Calcd for $C_{27}H_{40}O_4N_5$ 498.3080; Found 498.3074.


$^{1}$H NMR
NHBoc
N
N
O
N
N
O
2
[ppm]


$^{13}$C NMR
[ppm]

**2-(7,8-Dimethyl-3-octyl-2,4-dioxo-3,4-dihydrobenzo[g]pteridin-10(2H)-yl)ethan-1-aminium trifluoroacetate salt (F1):**

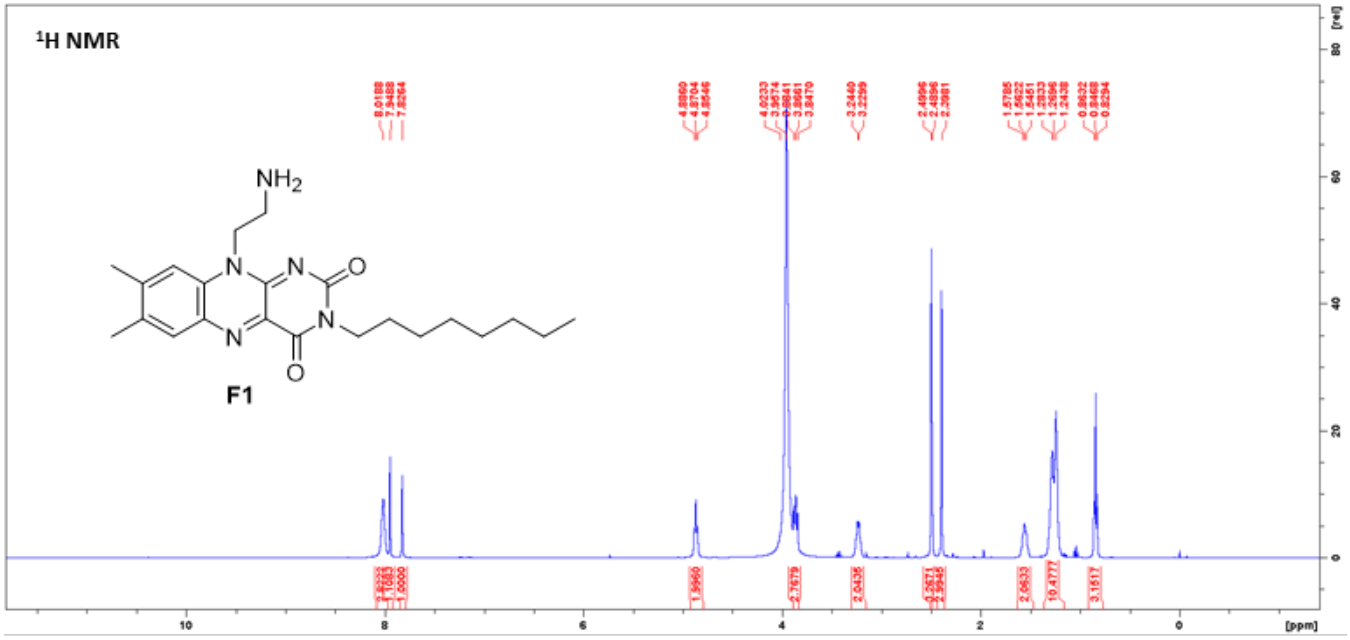


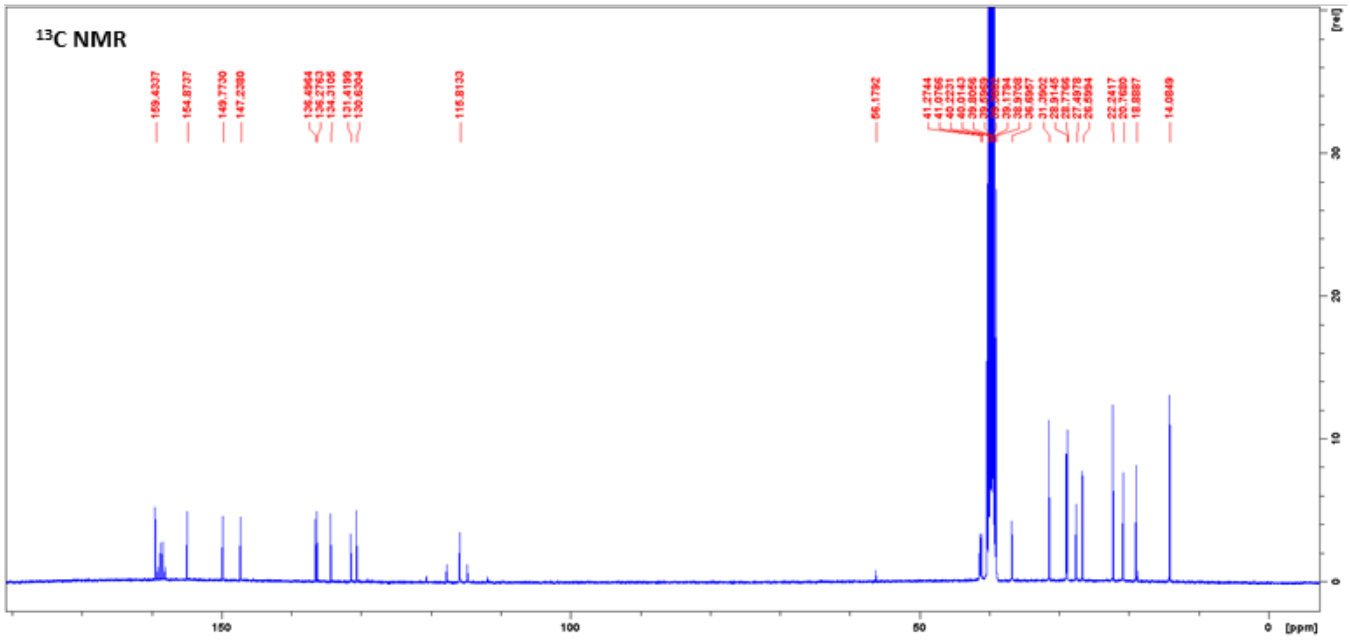

Flavin **2** (244 mg, 0.49 mmol) was dissolved in DCM (5 mL) and TFA (2 mL) was added dropwise. The resulting mixture was stirred at RT for 2 h (reaction completion judged by TLC) before the solvent was removed under reduced pressure. Excess TFA was removed using toluene (20 mL) and DCM (3 x 20 mL) co-evaporation to yield the TFA salt of flavin **F1** as a yellow solid (248 mg, 99%).

**$^{1}$H NMR** (400 MHz, DMSO-$d_6$) δ = 8.01 (br s, 2H, **-$NH_2$**), 7.95 (s, 1H, **H10**), 7.82 (s, 1H, **H8**), 4.87 (t, *J* = 6.3 Hz, 2H, **H12**), 3.87 (t, *J* = 7.4 Hz, 2H, **H11**), 3.23 (br m, 2H, **H13**), 2.50 (s, 3H, **H20**), 2.40 (s, 3H, **H21**), 1.56 (quint, *J* = 7.3 Hz, 2H, **H17**), 1.32-1.23 (m, 10H, **H14**/**15**/**16**/**18**/**19**), 0.85 (t, *J* = 6.8 Hz, 3H, **H22**) ppm.

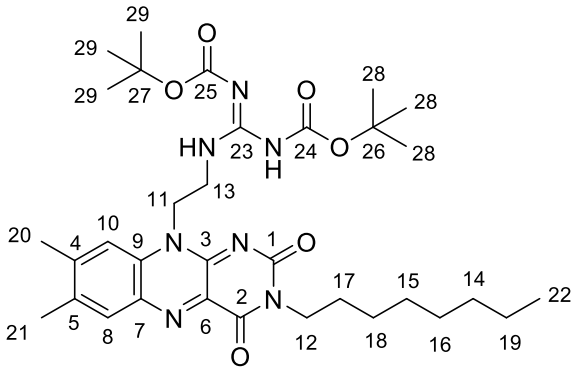
**$^{13}$C NMR** (100 MHz, DMSO-$d_6$) δ = 159.4 (**C1**), 154.9 (**C2**), 149.8 (**C3**), 147.2 (**C4**), 136.5 (**C5**), 136.3 (**C6**), 134.3 (**C7**), 131.4 (**C8**), 130.6 (**C9**), 115.8 (**C10**), 41.2 (**C11**), 41.0 (**C12**), 36.7 (**C13**), 31.4 (**C14**), 28.9 (**C15**), 28.8 (**C16**), 27.5 (**C17**), 26.6 (**C18**), 22.2 (**C19**), 20.7 (**C20**), 18.9 (**C21**), 14.1 (**C22**) ppm.

**HRMS (ESI)** m/z: $[M + H]^+$ Calcd for $C_{22}H_{32}O_2N_5$ 398.2556; Found 398.2564.


$^{1}$H NMR
NH2
N
N
O
N
N
O
F1
[ppm]


$^{13}$C NMR
159.4337
154.8737
149.7730
147.2380
136.4964
136.2763
134.3168
131.4199
130.6304
116.8133
56.1792
41.2744
41.0766
40.2231
40.0143
39.8056
39.5969
39.1794
38.9708
36.6957
31.3922
28.7166
27.4978
26.8994
22.2417
20.7480
18.8887
14.0849
150
100
50
0
[ppm]

**1,3-Di-Boc-2-(2-(7,8-dimethyl-3-octyl-2,4-dioxo-3,4-dihydrobenzo[g]pteridin-10(2H)-yl)ethyl)guanidine (3):**

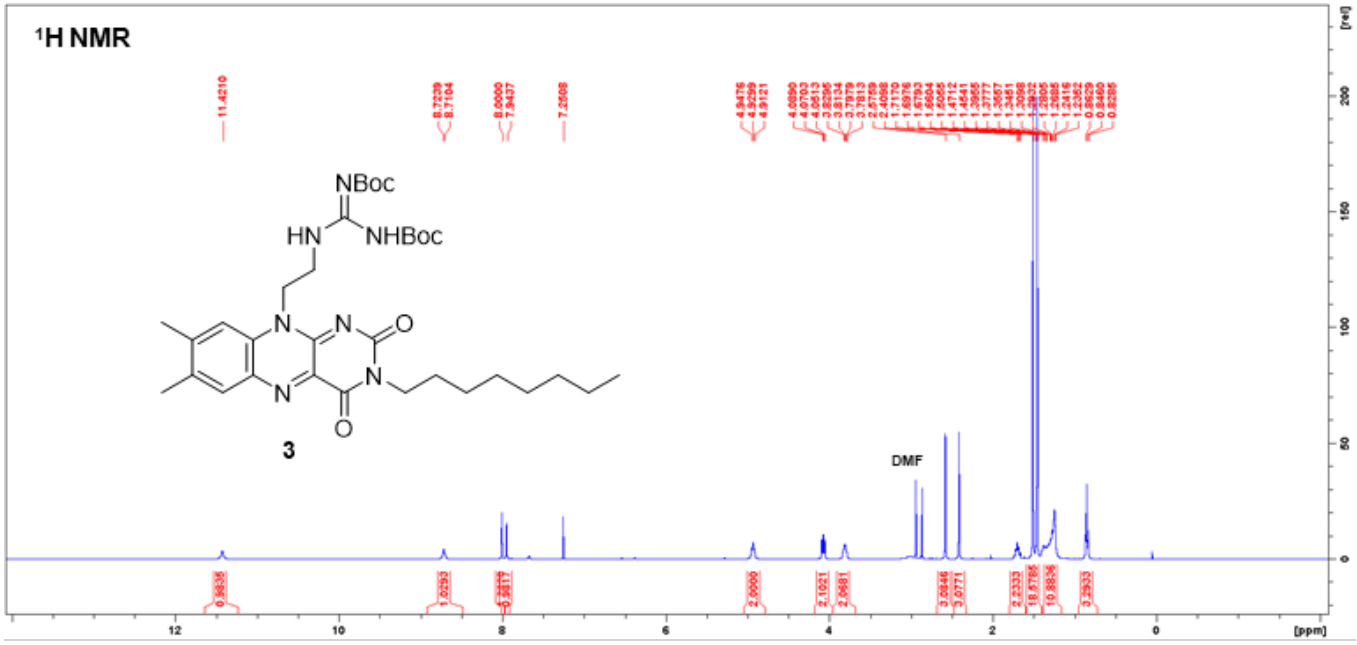


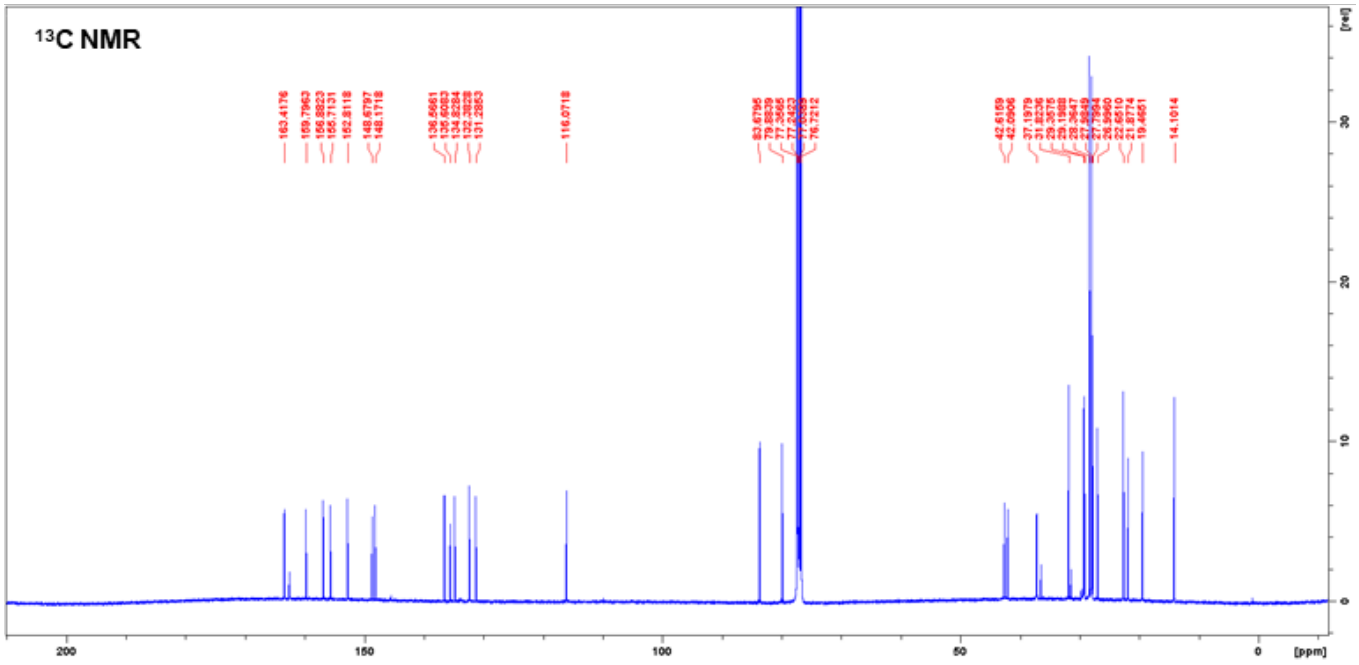
Flavin **2** (0.244 g, 0.49 mmol) was dissolved in DCM (5 mL) and TFA (2 mL) was added dropwise. The resulting mixture was stirred at RT for 2 h (reaction completion judged by TLC) before the solvent was removed under reduced pressure. Excess TFA was removed using toluene (3 x 20 mL) and DCM (3 x 20 mL) co-evaporation to yield flavin **1** which was then dissolved in anhydrous DMF (5 mL) under Ar atmosphere. DIPEA (0.17 mL, 0.98 mmol, 2.0 equiv.) was then added, followed by *N*,*N*′-di-Boc-1*H*-pyrazole-1-carboxamidine (152 mg, 0.49 mmol, 1.0 equiv). The mixture was then stirred for 18 h at RT under Ar atmosphere in the dark. The solvent was then removed under reduced pressure and the resulting residue was suspended in EtOAc (20 mL) and washed with water (3 x 20 mL) and brine (20 mL). The organic phase was then dried over $Na_2SO_4$, filtered, and concentrated under reduced pressure. The resulting yellow residue was purified by silica gel column chromatography (10% EtOAc/DCM) to yield flavin **3** as a yellow solid (282 mg, 90% over two steps).

**$^1$H NMR** (400 MHz, $CDCl_3$) δ = 11.42 (br s, 1H, -**NH**-), 8.72 (br s, 1H, **-NH**-), 8.00 (s, 1H, **H10**), 7.25 (s, 1H, **H8**), 4.93 (t, *J* = 7.0 Hz, 2H, **H12**), 4.07 (t, *J* = 7.5 Hz, 2H, **H11**), 3.80 (br m, 2H, **H13**), 2.58 (s, 3H, **H20**), 2.41 (s, 3H, **H21**), 1.70 (quint, *J* = 7.42 Hz, 2H, **H17**), 1.51 (s, 9H, **H28**), 1.45 (s, 9H, **H29**), 1.40-1.19 (m, 10H, **H14**/**15**/**16**/**18**/**19**), 0.85 (t, *J* = 6.9 Hz, 3H, **H22**) ppm.

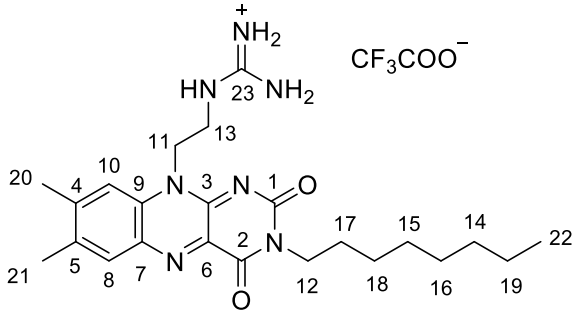
**$^{13}$C NMR** (100 MHz, $CDCl_3$) δ = 163.4 (**C23**), 159.8 (**C1**), 156.9 (**C24**), 155.7 (**C2**), 152.8 (**C25**), 148.7 (**C3**), 148.2 (**C4**), 136.6 (**C5**), 135.6 (**C6**), 134.8 (**C7**), 132.4 (**C8**), 131.3 (**C9**), 116.1 (**C10**), 83.7 (**C26**), 79.9 (**C27**), 42.2 (**C11**), 42.1 (**C12**), 37.2 (**C13**), 31.8 (**C14**), 29.4 (**C15**), 29.2 (**C16**), 28.4 (**C28**), 28.0 (**C29**), 27.8 (**C17**), 27.0 (**C18**), 22.7 (**C19**), 21.9 (**C20**), 19.5 (**C21**), 14.1 (**C22**) ppm.

**HRMS (ESI)** m/z: $[M + H]^+$ Calcd for $C_{33}H_{50}O_6N_7$ 640.3823; Found 640.3824.


¹H NMR
NBoc
HN
NHBoc
N
N
O
N
N
O
3
DMF
[ppm]


¹³C NMR
[ppm]

**1-(2-(7,8-Dimethyl-3-octyl-2,4-dioxo-3,4-dihydrobenzo[g]pteridin-10(2H)-yl)ethyl)guanidinium trifluoroacetate salt (F2):**

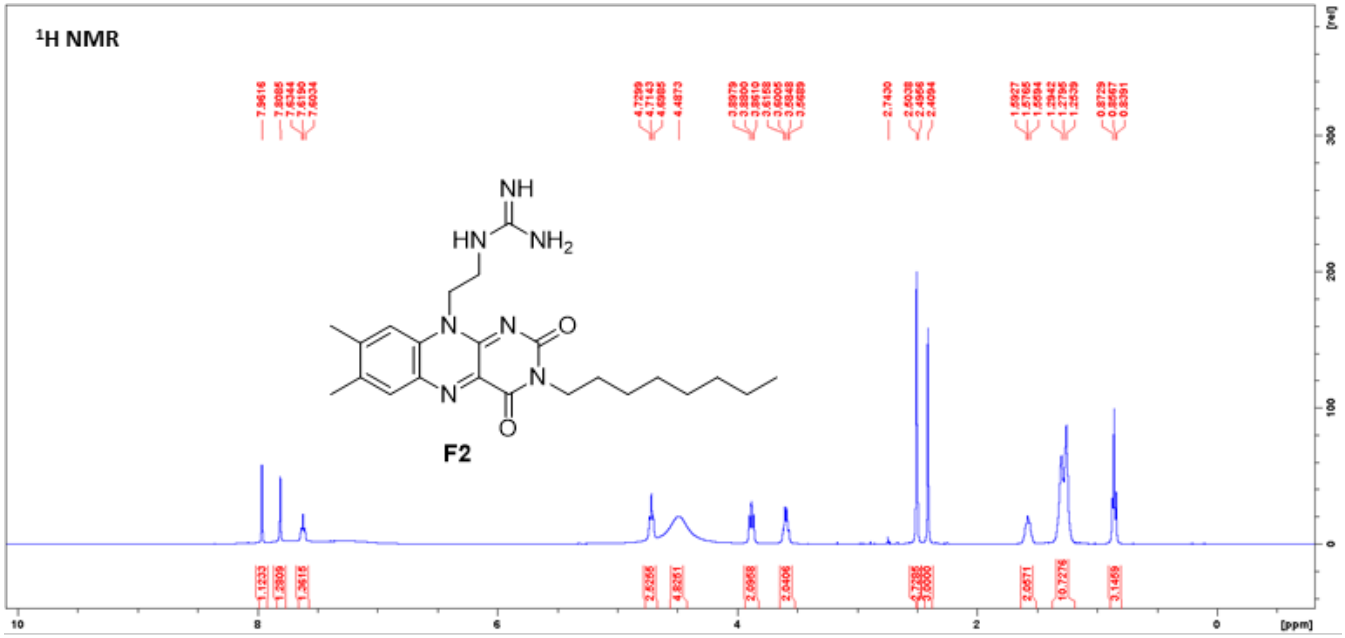


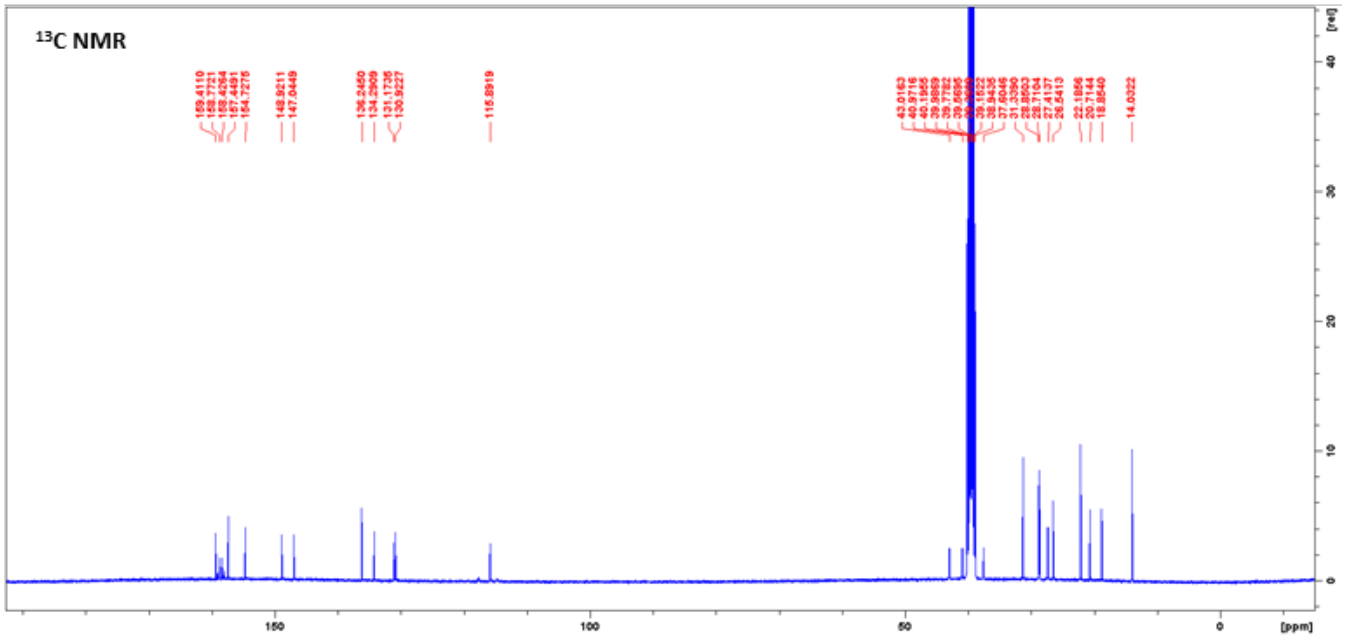
Flavin **3** (272 mg, 0.43 mmol) was dissolved in DCM (4 mL) and TFA (1.5 mL) was added dropwise. The resulting mixture was stirred at RT for 2 h (reaction completion judged by TLC) before the solvent was removed under reduced pressure. Excess TFA was removed using toluene (20 mL) and DCM (3 x 20 mL) co-evaporation to yield the TFA salt of flavin **F2** as a yellow solid (235 mg, 99%).

**$^{1}$H NMR** (400 MHz, DMSO-$d_6$) δ = 7.96 (s, 1H, **H8**), 7.81 (s, 1H, **H10**), 7.61 (br t, *J* = 6.2 Hz, 1H, -**NH**-) 4.71 (t, *J* = 6.3 Hz, 2H, **H12**), 4.48 (br s, 3H, =**NH**, -**$NH_2$**), 3.88 (t, *J* = 7.4 Hz, 2H, **H11**), 3.59 (br m, 2H, **H13**), 2.50 (s, 3H, **H20**), 2.41 (s, 3H, **H21**), 1.58 (br m, *J* = 7.42 Hz, 2H, **H17**), 1.33-1.23 (m, 10H, **H14**/**15**/**16**/**18**/**19**), 0.86 (t, *J* = 6.8 Hz, 3H, **H22**) ppm.

**$^{13}$C NMR** (100 MHz, DMSO-$d_6$) δ = 159.4 (**C1**), 157.4 (**C23**), 154.7 (**C2**), 148.9 (**C3**), 147.0 (**C4**), 136.2 (**C5, C6**), 134.3 (**C7**), 131.2 (**C8**), 130.9 (**C9**), 115.9 (**C10**), 43.0 (**C11**), 41.0 (**C12**), 37.6 (**C13**), 31.3 (**C14**), 28.9 (**C15**), 28.7 (**C16**), 27.4 (**C17**), 26.5 (**C18**), 22.2 (**C19**), 20.7 (**C20**), 18.9 (**C21**), 14.0 (**C22**) ppm.

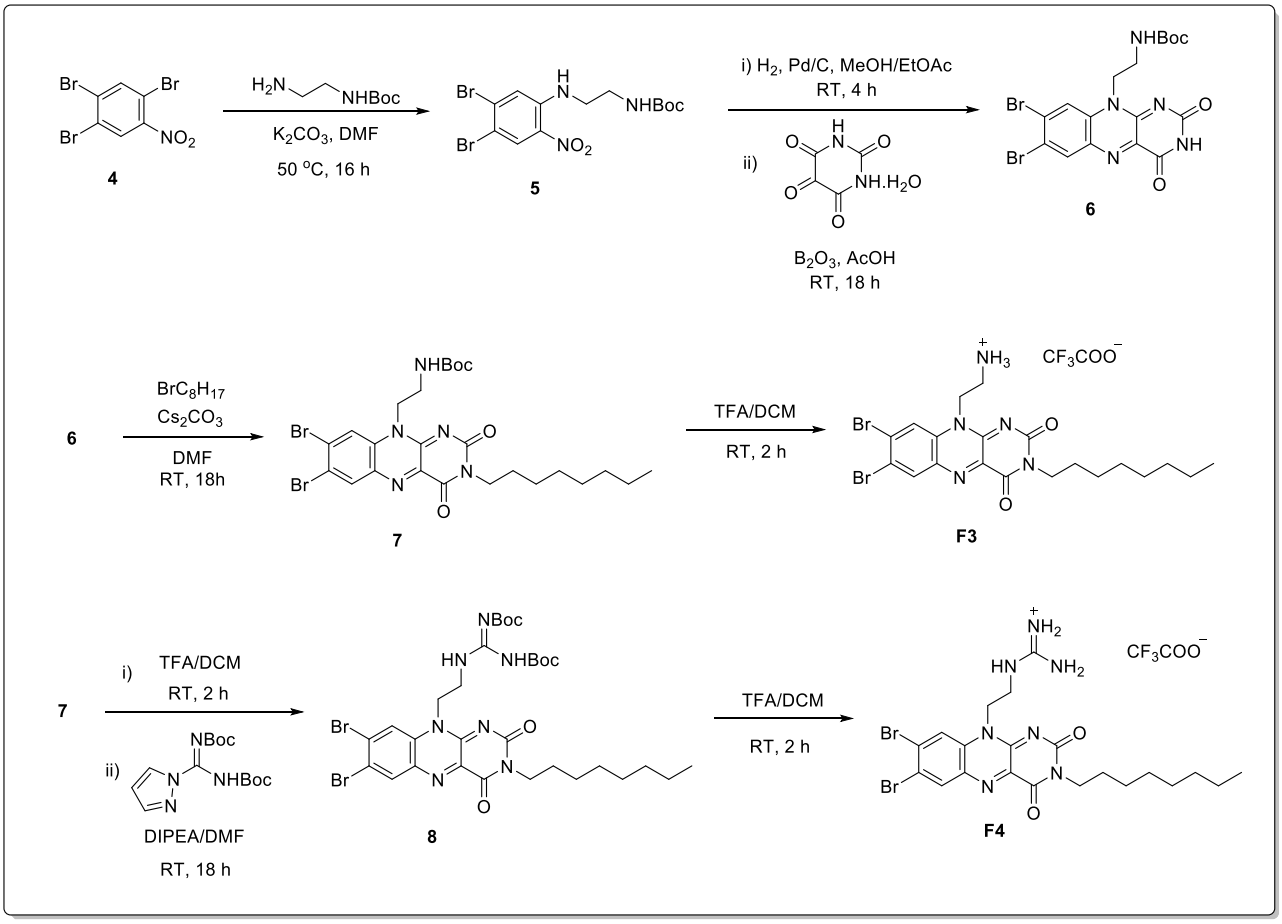
**HRMS (ESI)** m/z: $[M + H]^+$ Calcd for $C_{23}H_{34}O_2N_7$ 440.2774; Found 440.2783.


$^{1}$H NMR
NH
HN
NH$_2$
N
N
O
N
N
O
F2
[ppm]


$^{13}$C NMR
[ppm]

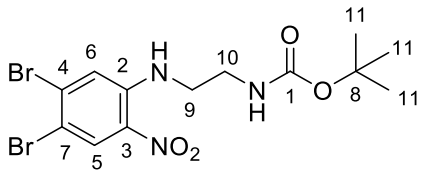
### *3.4 Flavins F3 and F4*

1,2,4-Tribromo-5-nitrobenzene (**4**) was prepared as previously reported by Legrand *et al.*>




**Scheme S2:** Synthesis of flavins **F3** and **F4.**

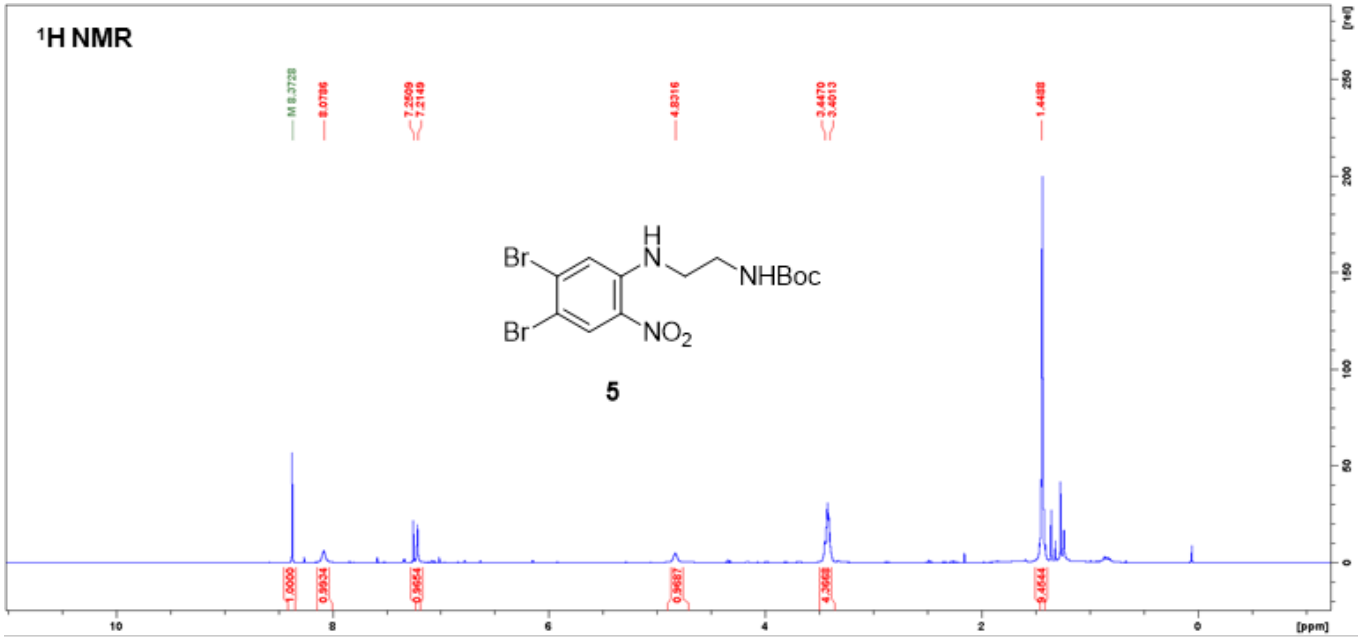
***tert*-Butyl (2-((4,5-dibromo-2-nitrophenyl)amino)ethyl)carbamate (5):**

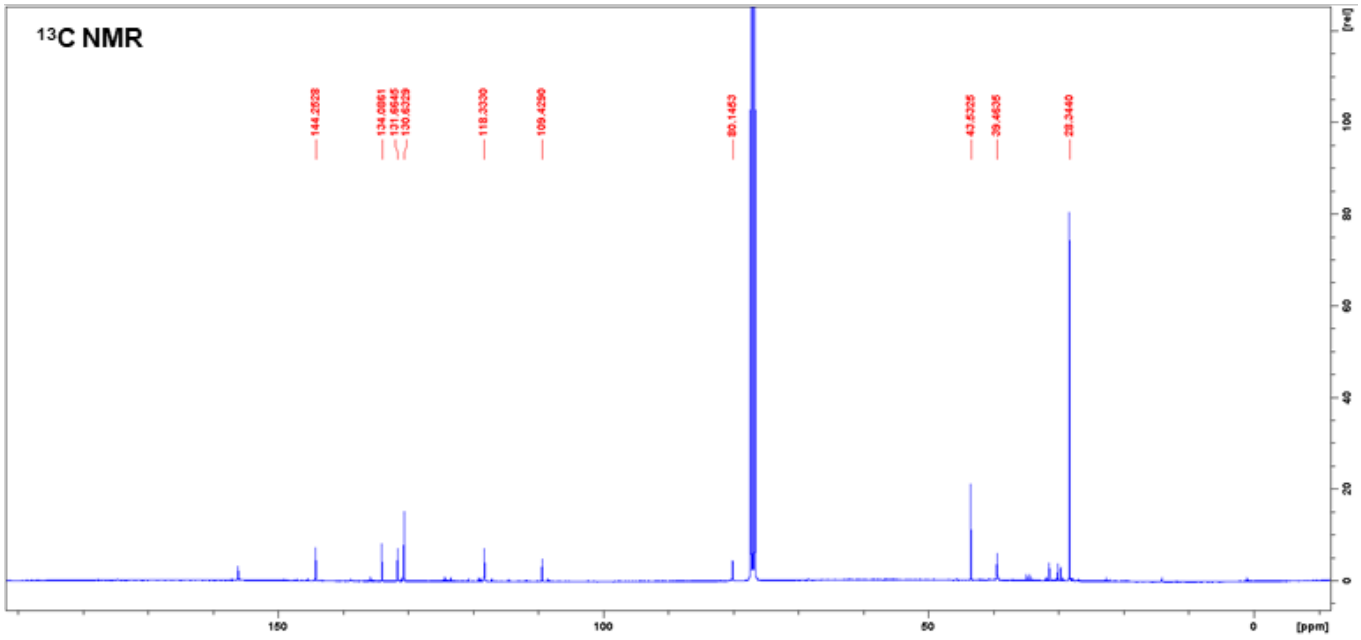



1,2,4-Tribromo-5-nitrobenzene (**4**) (1.00 g, 2.78 mmol, 1.0 equiv.) and anhydrous $K_2CO_3$ (576 mg, 4.17 mmol, 1.5 equiv.) were suspended in anhydrous DMF (25 mL) before adding a solution of *tert*-butyl (2-aminoethyl)carbamate (1.70 g, 10.61 mmol, 5.0 equiv.) in anhydrous DMF (5 mL) dropwise at RT under Ar atmosphere. The resulting mixture was then stirred at 50 $^oC$ for 18 h. The solvent was then removed under reduced pressure and the resulting residue was suspended in EtOAc (20 mL) and washed with water (3 x 20 mL) and brine (20 mL). The organic phase was then dried over $Na_2SO_4$, filtered, and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (5-10% EtOAc/Cyclohexane) to yield **5** as an orange solid (785 mg, 64%).

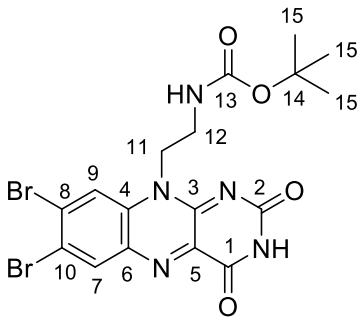
**$^1$H NMR** (400 MHz, $CDCl_3$) δ = 8.37 (s, 1H, **H5**), 8.08 (br s, 1H, -**NH**-), 7.21 (s, 1H, H6), 4.83 (br s, 1H, Ar-**NH**-), 3.45-3.40 (br m, 4H, **H9**, **H10**), 1.45 (s, 9H, **H11**) ppm.
**$^{13}$C NMR** (100 MHz, $CDCl_3$) δ = 156.2 (**C1**), 144.3 (**C23**), 134.1 (**C2**), 131.7 (**C3**), 130.6 (**C4**), 118.3 (**C5**), 109.4 (**C6**), 80.1 (**C7**), 43.5 (**C8**), 39.5 (**C9**), 28.3 (**C10**) ppm.

**HRMS (ESI)** m/z: $[M + H]^+$ Calcd for $C_{13}H_{17}O_4N_3Br_2Na$ 459.9484; Found 459.9470.


¹H NMR
Br
Br
H
N
NHBoc
NO2
5
[ppm]


¹³C NMR
[ppm]

***tert*-Butyl (2-(7,8-dibromo-2,4-dioxo-3,4-dihydrobenzo[*g*]pteridin-10(2*H*)-yl)ethyl)carbamate (6):**

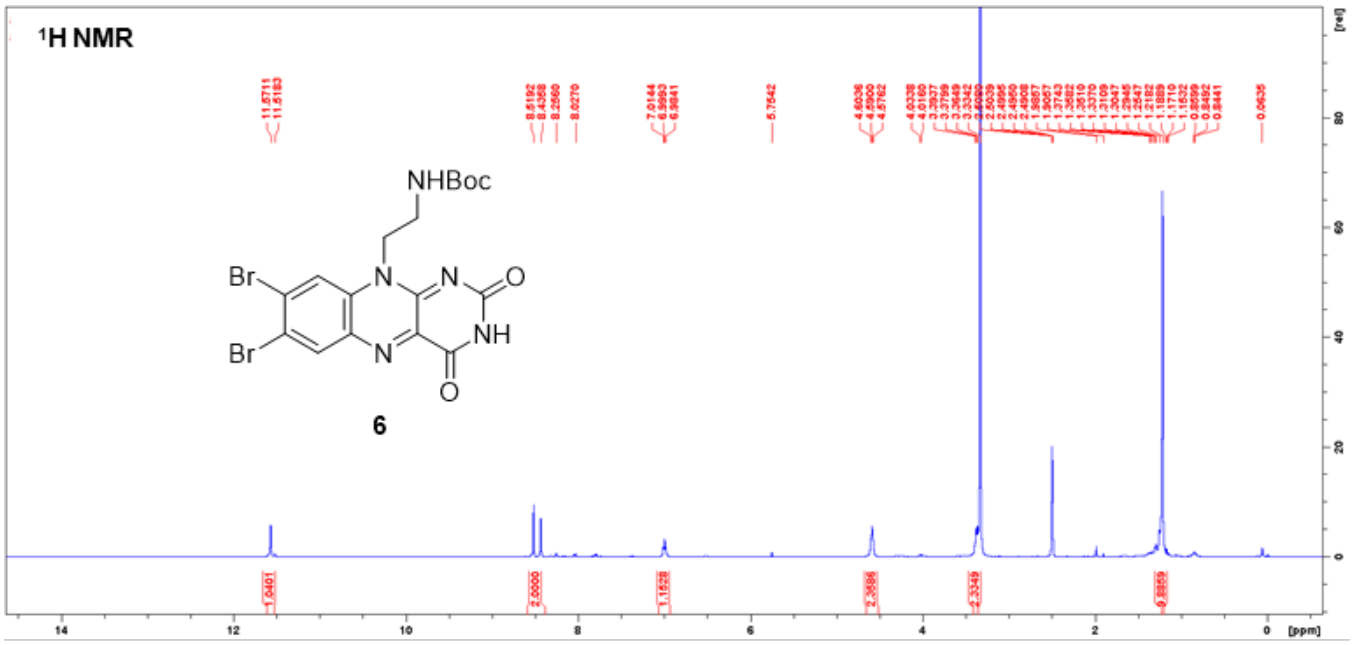


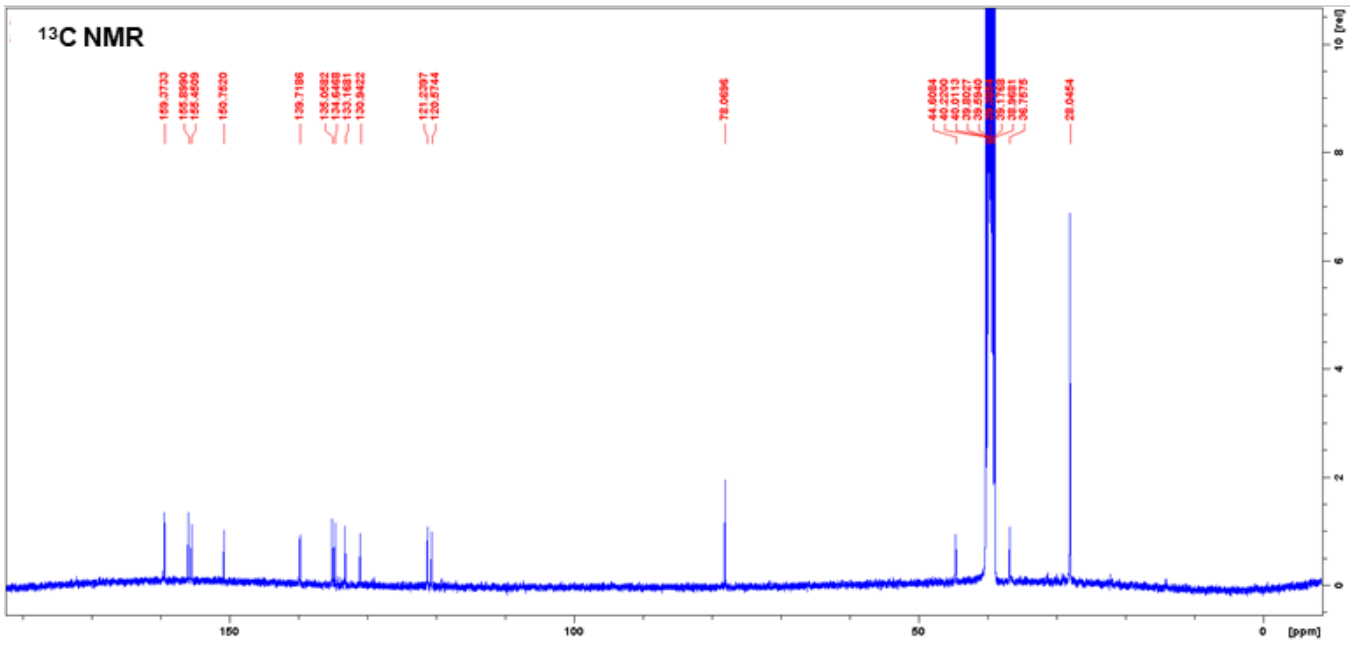

*tert*-Butyl (2-((4,5-dibromo-2-nitrophenyl)amino)ethyl)carbamate (**5**) (785 mg, 1.78 mmol, 1.0 equiv.) was dissolved in MeOH/EtOAc (1:1 v/v, 20 mL) and system purged with Ar. Pd/C (50 mg) was then added and the atmosphere was replaced with $H_2$ gas (2 x balloons). The resulting mixture was stirred for 4 h (reaction completion judged by TLC) at RT before being filtered through Celite and concentrated under reduced pressure. The resulting red residue was then dissolved in glacial acetic acid (20 mL) under Ar atmosphere. Subsequently, $B_2O_3$ (248 mg, 3.56 mmol, 2.0 equiv.) was added, followed by alloxan monohydrate (285 mg, 1.78 mmol, 1.0 equiv) and the resulting reaction mixture was stirred for 18 h under Ar atmosphere in the dark. The mixture was then partitioned between EtOAc (20 mL) and water (20 mL), and the organic phase was washed with brine (3 x 20 mL), dried over $Na_2SO_4$, filtered and concentrated under reduced pressure. The orange residue was then purified by silica gel column chromatography (1% EtOH/EtOAc) to yield flavin **6** as an orange solid (313 mg, 34% over two steps).

**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ = 11.57 (s, 1H, Ar-**NH**), 8.52 (s, 1H, **H7**), 8.44 (s, 1H, **H9**), 7.00 (br t, *J* = 6.0 Hz, 1H, -**NH**-), 4.59 (t, *J* = 5.2 Hz, 2H, **H11**), 3.36 (br m, 2H, **H12**), 1.22 (s, 9H, **H15**) ppm.

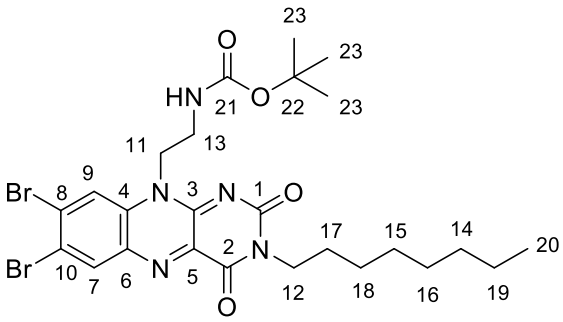
**$^{13}$C NMR** (100 MHz, DMSO-$d_6$) δ = 159.4 (**C1**), 155.9 (**C13**), 155.4 (**C2**), 150.8 (**C3**), 139.7 (**C4**), 135.1 (**C5**), 134.6 (**C6**), 133.2 (**C7**), 130.9 (**C8**), 121.2 (**C9**), 120.6 (**C10**), 78.1 (**C14**), 44.6 (**C11**), 36.8 (**C12**), 28.0 (**C15**) ppm.

**HRMS (ESI)** m/z: $[M + H]^+$ Calcd for $C_{17}H_{18}O_4N_5Br_2$ 513.9726; Found 513.9739.


$^{1}$H NMR
NHBoc
Br
Br
N
N
N
O
NH
O
6
[ppm]


$^{13}$C NMR
[ppm]

***tert*-Butyl (2-(7,8-dibromo-3-octyl-2,4-dioxo-3,4-dihydrobenzo[*g*]pteridin-10(2*H*)-yl)ethyl)carbamate (7):**

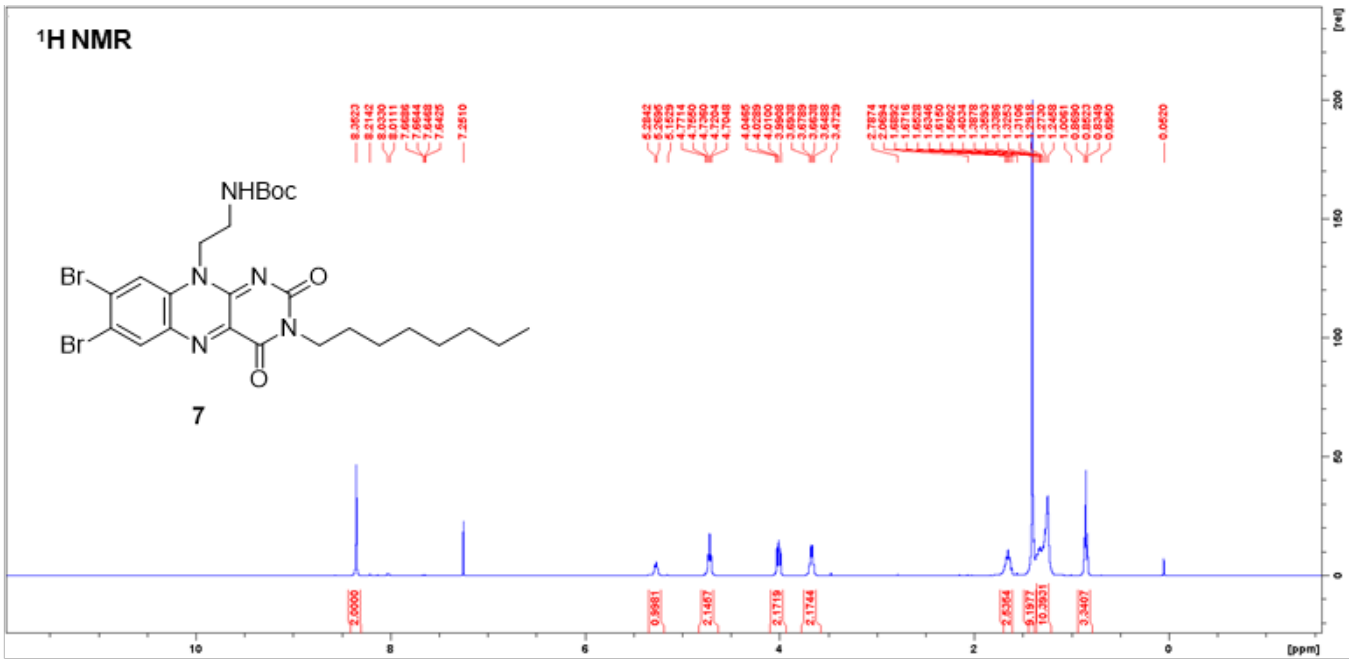


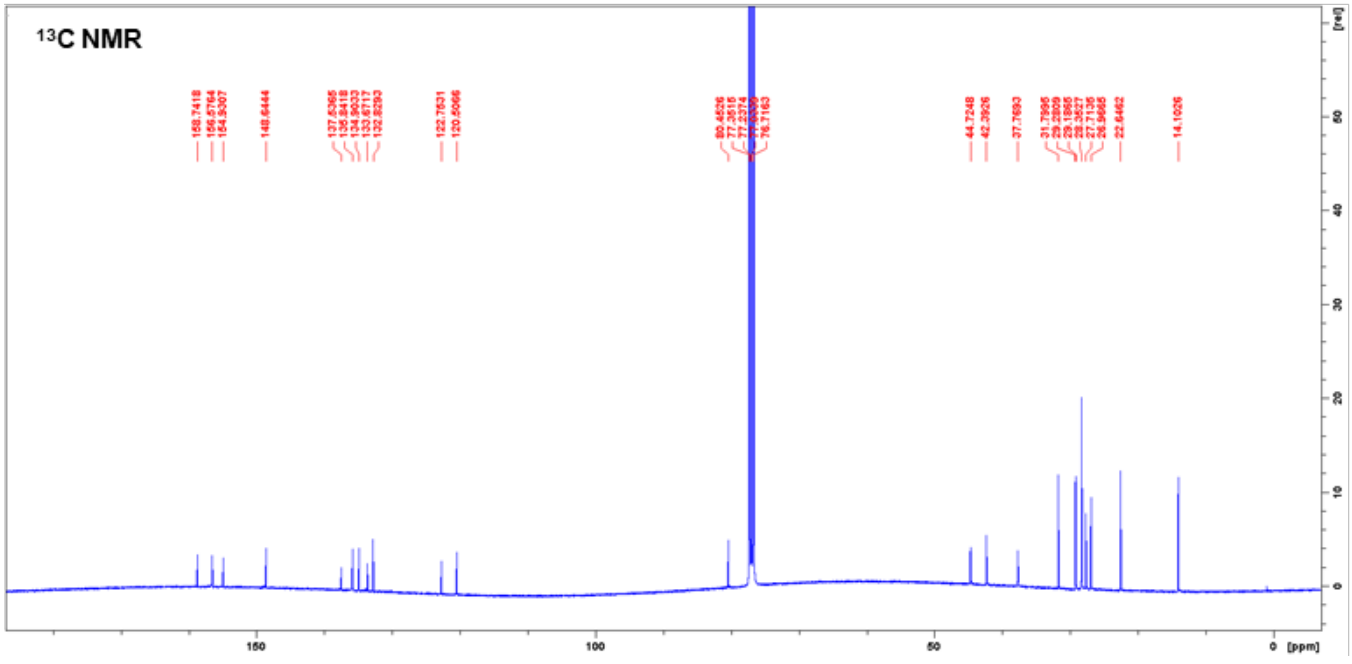
Flavin **6** (504 mg, 0.98 mmol, 1.0 equiv.) and 1-bromooctane (944 mg, 0.86 mL, 4.89 mmol, 5.0 equiv.) were dissolved in anhydrous DMF (20 mL) under Ar atmosphere. $Cs_2CO_3$ (478 mg, 1.47 mmol, 1.5 equiv.) was then added and the resulting mixture was stirred for 18 h at RT under Ar in the dark. The solvent was then removed under reduced pressure and the resulting residue was suspended in DCM (20 mL) and washed with water (3 x 20 mL) and brine (20 mL). The organic phase was then dried over $Na_2SO_4$, filtered, and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (10-20% EtOAc/DCM) to yield flavin **7** as an orange solid (444 mg, 72%).

**$^1$H NMR** (400 MHz, $CDCl_3$) δ = 8.35 (s, 2H, **H7**, **H9**), 5.27 (br t, *J* = 5.7 Hz, 1H, -**NH**-), 4.72 (t, *J* = 6.2 Hz, 2H, **H12**), 4.01 (t, *J* = 7.6 Hz, 2H, **H11**), 3.67 (q, *J* = 6.0 Hz, 2H, **H13**), 1.65 (quint, *J* = 7.4 Hz, 2H, **H17**), 1.40 (s, 9H, **H23**), 1.36-1.24 (m, 10H, **H14**/**15**/**16**/**18**/**19**), 0.85 (t, *J* = 6.8 Hz, 3H, **H20**) ppm.

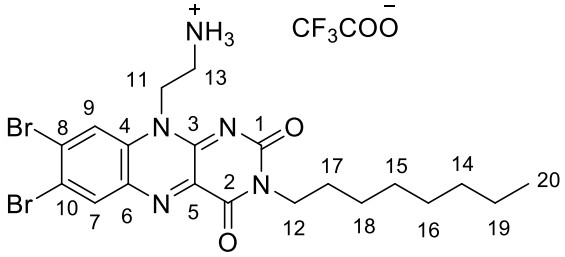
**$^{13}$C NMR** (100 MHz, $CDCl_3$) δ = 158.7 (**C1**), 156.6 (**C21**), 154.9 (**C2**), 148.6 (**C3**), 137.5 (**C4**), 135.8 (**C5**), 134.9 (**C6**), 133.7 (**C7**), 132.8 (**C8**), 122.8 (**C9**), 120.5 (**C10**), 80.5 (**C22**), 44.7 (**C11**), 42.4 (**C12**), 38.3 (**C13**), 31.8 (**C14**), 29.3 (**C15**), 29.2 (**C16**), 28.4 (**C23**), 27.7 (**C17**), 27.0 (**C18**), 22.6 (**C19**), 14.1 (**C20**) ppm.

**HRMS (ESI)** m/z: $[M + H]^+$ Calcd for $C_{25}H_{34}O_4N_5Br_2$ 626.0978; Found 626.0980.


$^{1}$H NMR
NHBoc
Br
Br
N
N
O
O
7
[ppm]


$^{13}$C NMR
[ppm]

**2-(7,8-Dibromo-3-octyl-2,4-dioxo-3,4-dihydrobenzo[g]pteridin-10(2H)-yl)ethan-1-aminium trifluoroacetate salt (F3):**

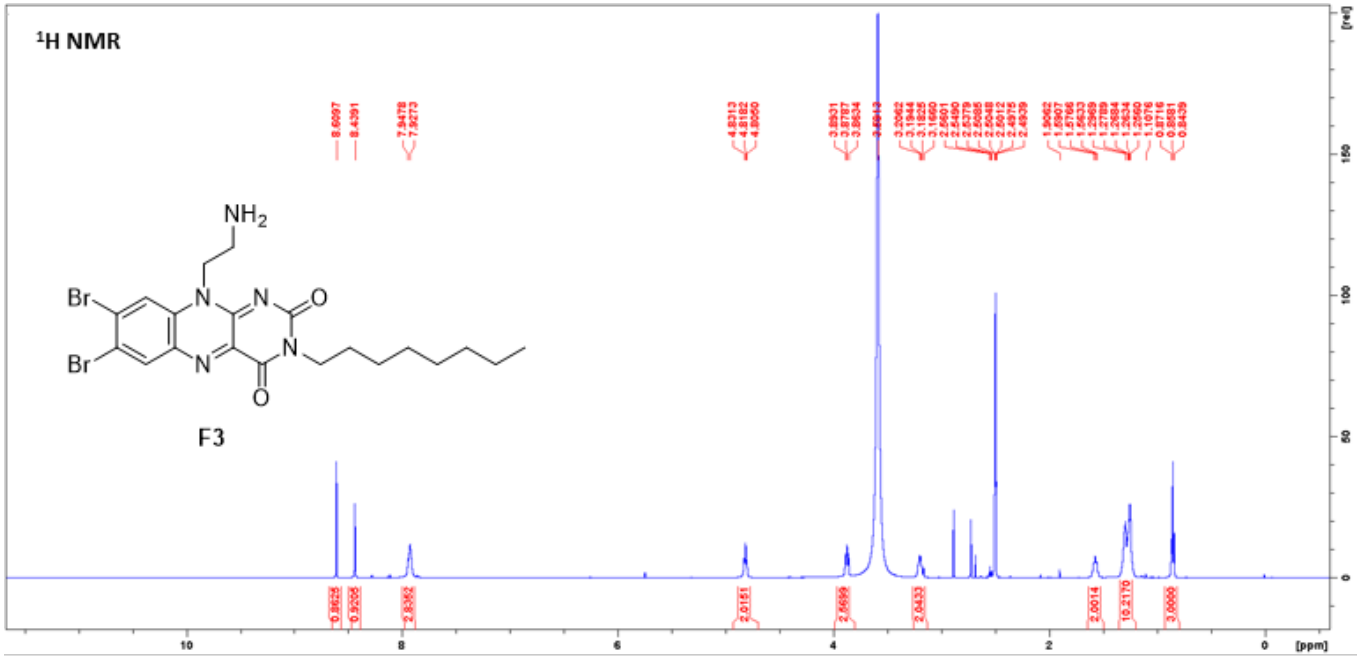


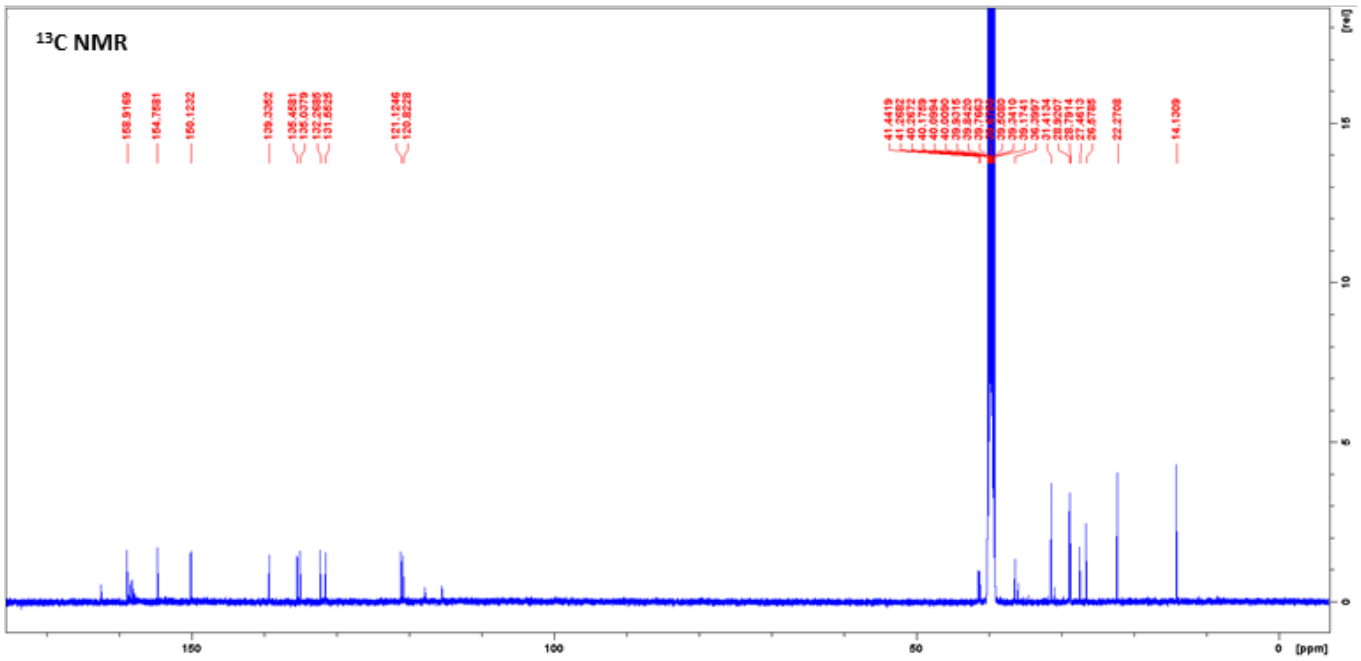
Flavin **7** (212 mg, 0.34 mmol) was dissolved in DCM (4 mL) and TFA (1.5 mL) was added dropwise. The resulting mixture was stirred at RT for 2 h (reaction completion judged by TLC) before the solvent was removed under reduced pressure. Excess TFA was removed using toluene (20 mL) and DCM (3 x 20 mL) co-evaporation to yield the TFA salt of flavin **F3** as a yellow solid (215 mg, 99%).

**$^{1}$H NMR** (400 MHz, DMSO-$d_6$) δ = 8.61 (s, 1H, **H7**), 8.35 (s, 2H, **H9**), 7.93 (br m, *J* = 5.7 Hz, 2H, -**$NH_2$**), 4.82 (t, *J* = 6.6 Hz, 2H, **H12**), 3.88 (t, *J* = 7.4 Hz, 2H, **H11**), 3.67 (br m, 2H, **H13**), 1.57 (quint, *J* = 7.3 Hz, 2H, **H17**), 1.30-1.23 (m, 10H, **H14**/**15**/**16**/**18**/**19**), 0.86 (t, *J* = 6.9 Hz, 3H, **H20**) ppm.

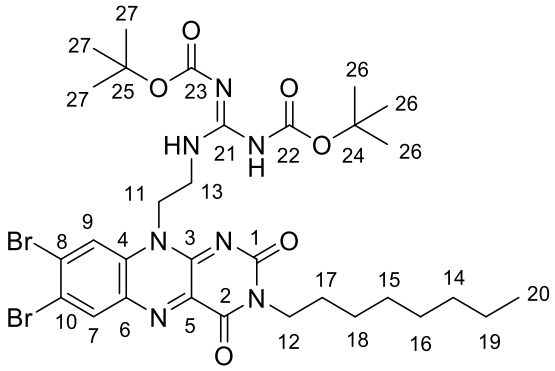
**$^{13}$C NMR** (100 MHz, DMSO-$d_6$) δ = 158.9 (**C1**), 154.8 (**C2**), 150.1 (**C3**), 139.3 (**C4**), 135.4 (**C5**), 135.0 (**C6**), 132.3 (**C7**), 131.6 (**C8**), 121.1 (**C9**), 120.8 (**C10**), 41.4 (**C11**), 41.3 (**C12**), 36.4 (**C13**), 31.4 (**C14**), 28.9 (**C15**), 28.8 (**C16**), 27.5 (**C17**), 26.6 (**C18**), 22.3 (**C19**), 14.1 (**C20**) ppm.

**HRMS (ESI)** m/z: $[M + H]^+$ Calcd for $C_{20}H_{26}O_2N_5Br_2$ 526.0453; Found 526.0455.


$^{1}$H NMR
NH2
Br
Br
N
N
O
O
F3
[ppm]


$^{13}$C NMR
[ppm]

**1,3-Di-Boc-2-(2-(7,8-dibromo-3-octyl-2,4-dioxo-3,4-dihydrobenzo[g]pteridin-10(2H)-yl)ethyl)guanidine (8):**

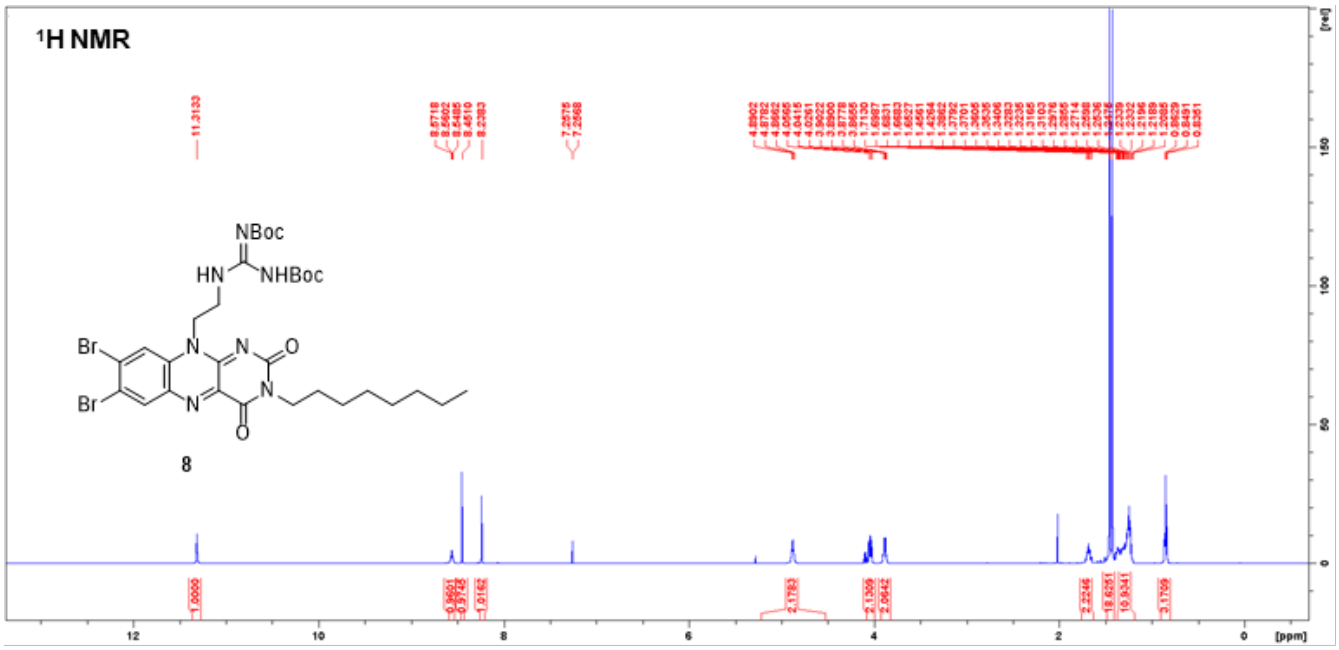


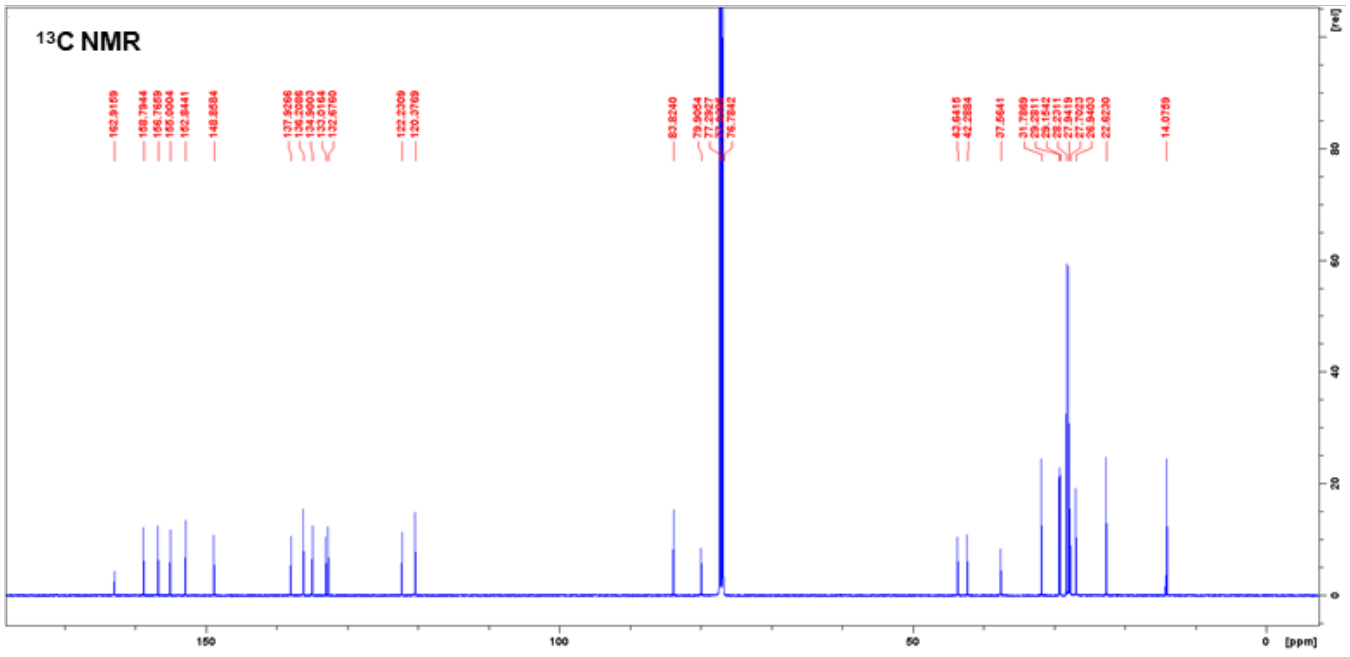
Flavin **7** (270 mg, 0.43 mmol) was dissolved in DCM (4 mL) and TFA (1.5 mL) was added dropwise. The resulting mixture was stirred at RT for 2 h (reaction completion judged by TLC) before the solvent was removed under reduced pressure. Excess TFA was removed using toluene (3 x 20 mL) and DCM (3 x 20 mL) co-evaporation to yield flavin **3** which was then dissolved in anhydrous DMF (5 mL) under Ar atmosphere. DIPEA (0.15 mL, 0.86 mmol, 2.0 equiv.) was then added, followed by *N*,*N*′-di-Boc-1*H*-pyrazole-1-carboxamidine (133 mg, 0.43 mmol, 1.0 equiv). The mixture was then stirred for 18 h at RT under Ar atmosphere in the dark. The solvent was then removed under reduced pressure and the resulting residue was suspended in DCM (20 mL) and washed with water (3 x 20 mL) and brine (20 mL). The organic phase was then dried over $Na_2SO_4$, filtered, and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (10% EtOAc/DCM) to yield flavin **8** as an orange solid (264 mg, 80% over two steps).

**$^1$H NMR** (400 MHz, $CDCl_3$) δ = 11.3 (br s, 1H, -**NH**-), 8.57 (br t, *J* = 5.8 Hz 1H, **-NH**-), 8.45 (s, 1H, **H7**), 8.24 (s, 1H, **H9**), 4.88 (t, *J* = 6.0 Hz, 2H, **H12**), 4.04 (t, *J* = 7.6 Hz, 2H, **H11**), 3.88 (q, *J* = 6.1 Hz, 2H, **H13**), 1.68 (quint, *J* = 7.5 Hz, 2H, **H17**), 1.46 (s, 9H, **H26**), 1.43 (s, 9H, **H27**), 1.40-1.21 (m, 10H, **H14**/**15**/**16**/**18**/**19**), 0.85 (t, *J* = 7.0 Hz, 3H, **H22**) ppm.

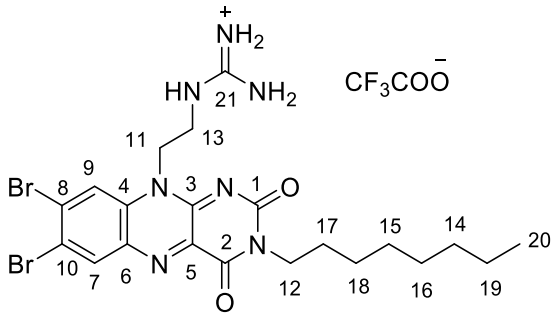
**$^{13}$C NMR** (100 MHz, $CDCl_3$) δ = 162.9 (**C21**), 158.8 (**C1**), 156.8 (**C23**), 155.0 (**C2**), 152.8 (**C22**), 148.9 (**C3**), 137.9 (**C4**), 136.2 (**C5**), 134.9 (**C6**), 133.0 (**C7**), 132.7 (**C8**), 122.2 (**C9**), 120.4 (**C10**), 83.8 (**C24**), 79.9 (**C25**), 43.6 (**C11**), 42.3 (**C12**), 37.6 (**C13**), 31.8 (**C14**), 29.3 (**C15**), 29.2 (**C16**), 28.3 (**C26**), 28.0 (**C27**), 27.7 (**C17**), 26.9 (**C18**), 22.6 (**C19**), 14.1 (**C20**) ppm.

**HRMS (ESI)** m/z: $[M + H]^+$ Calcd for $C_{31}H_{44}O_6N_7Br_2$ 768.1720; Found 768.1720.


$^{1}$H NMR
NBoc
HN
NHBoc
Br
Br
N
N
O
O
N
N
O
8
[ppm]


$^{13}$C NMR
[ppm]

**1-(2-(7,8-Dibromo-3-octyl-2,4-dioxo-3,4-dihydrobenzo[g]pteridin-10(2H)-yl)ethyl)guanidinium trifluoroacetate (F4):**

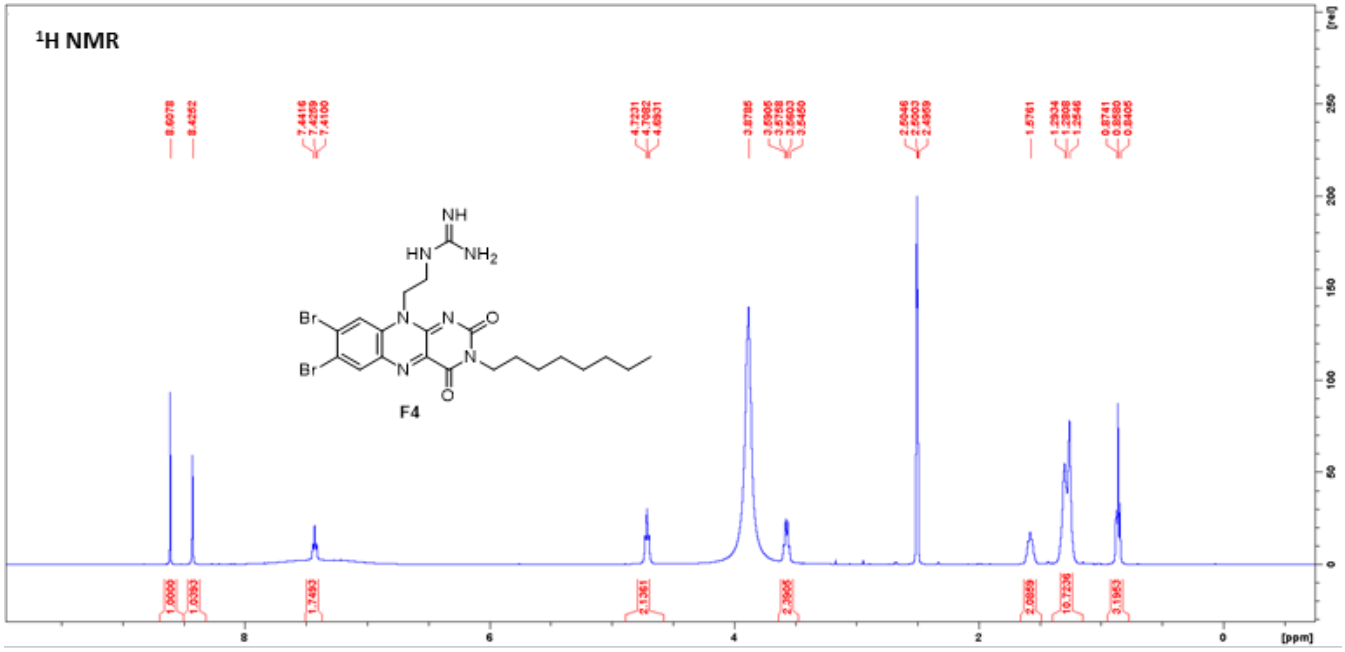


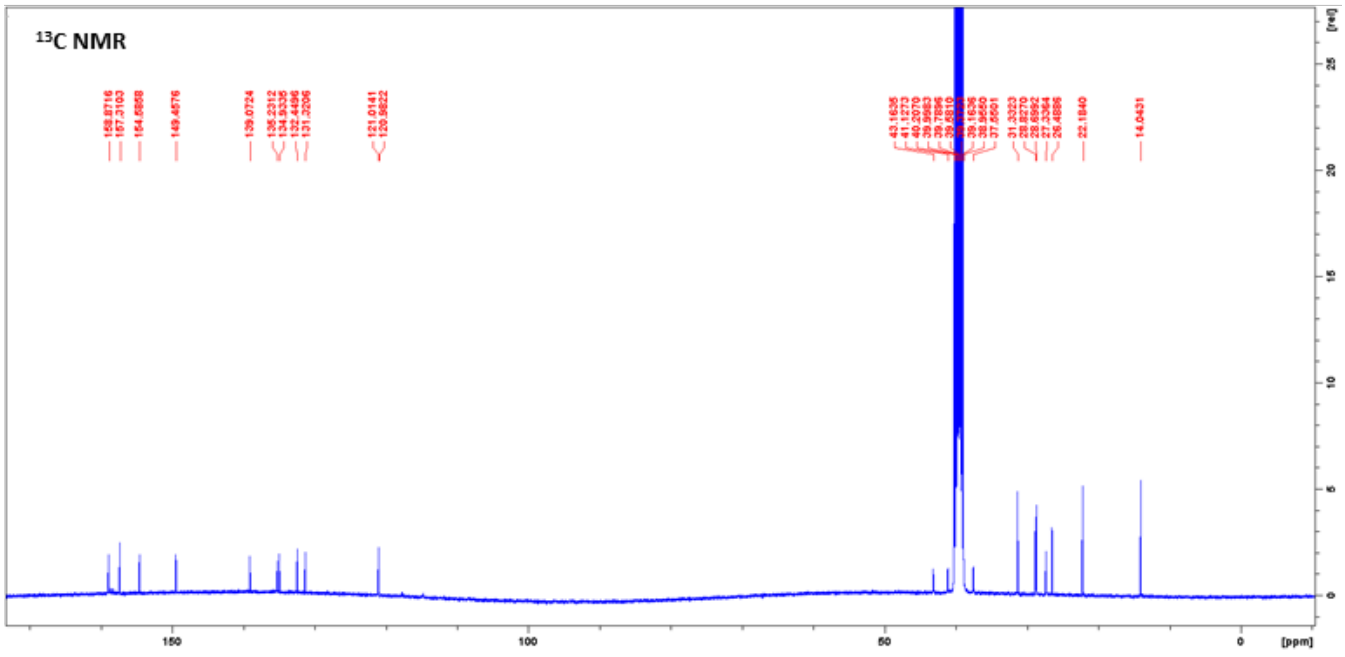
Flavin **8** (264 mg, 0.34 mmol) was dissolved in DCM (4 mL) and TFA (1.5 mL) was added dropwise. The resulting mixture was stirred at RT for 2 h (reaction completion judged by TLC) before the solvent was removed under reduced pressure. Excess TFA was removed using toluene (20 mL) and DCM (3 x 20 mL) co-evaporation to yield the TFA salt of flavin **F4** as a yellow solid (232 mg, 99%).

**$^{1}$H NMR** (400 MHz, DMSO-$d_6$) δ = 8.61 (s, 1H, **H7**), 8.42 (s, 1H, **H9**), 7.43 (br t, *J* = 6.3 Hz, 1H, -**NH**-) 4.71 (t, *J* = 6.0 Hz, 2H, **H12**), 3.87 (br s, 3H, =**NH**, -**$NH_2$**), 3.57 (t, *J* = 6.1 Hz, 2H, **H11**), 3.59 (br m, 2H, **H13**), 2.50 (s, 3H, **H20**), 2.41 (s, 3H, **H21**), 1.58 (br m, *J* = 7.42 Hz, 2H, **H17**), 1.33-1.23 (m, 10H, **H14**/**15**/**16**/**18**/**19**), 0.86 (t, *J* = 6.7 Hz, 3H, **H22**) ppm.

**$^{13}$C NMR** (100 MHz, DMSO-$d_6$) δ = 158.9 (**C1**), 157.3 (**C21**), 154.6 (**C2**), 149.5 (**C3**), 139.1 (**C4**), 135.2 (**C5**), 134.9 (**C6**), 132.4 (**C7**), 131.3 (**C8**), 121.0 (**C9**), 120.9 (**C10**), 43.2 (**C11**), 41.1 (**C12**), 37.5 (**C13**), 31.3 (**C14**), 28.8 (**C15**), 28.7 (**C16**), 27.3 (**C17**), 26.5 (**C18**), 22.2 (**C19**), 14.0 (**C20**) ppm.

**HRMS (ESI)** m/z: $[M + H]^+$ Calcd for $C_{21}H_{28}O_2N_7Br_2$ 568.0671; Found 568.0669.


$^{1}$H NMR
NH
HN
NH$_2$
Br
Br
N
O
F4
[ppm]


$^{13}$C NMR
[ppm]

## 4 Fluorescence Quantum Yield Calculations

The fluorescence quantum yields ($\Phi_F$) for **F1-4** were determined by preparing a series of dilutions in DMSO ranging in absorbance values from 0.02 to 0.10 and excited at their respective absorbance maxima (see **Table S1**).> The emission peak was then integrated and plotted against the absorbance value (see **Figure S1**). Riboflavin (from *Eremothecium ashbyii*, ≥98%, Sigma) was chosen as the reference $\phi_F$ for calculations ($\Phi_F$= 0.226 ± 0.01 in DMSO)[4] according to equation (1):

$$\Phi_{\mathrm{F;Sample}} = \Phi_{\mathrm{F;Ref}} \left(\frac{Slope_{Sample}}{Slope_{Ref}}\right) \left(\frac{\eta^2_{Sample}}{\eta^2_{Ref}}\right) \quad (1)$$

Where Slope = gradient from the plot of integrated emission peak vs. absorption value and η = refractive index of the solvent.

**Table S1:** Spectroscopic details of flavins **F1-4** and reference compound riboflavin used for $\phi_F$ calculations in DMSO.

| Flavin | ε ($M^{-1}cm^{-1}$) | $\lambda_{ex}$ (nm) | $\lambda_{em}$ (nm) |
|---|---|---|---|
| **Riboflavin** | 11718.41 | 447 | 513 |
| **F1** | 10368.79 | 444 | 507 |
| **F2** | 9838.05 | 446 | 512 |
| **F3** | 9541.26 | 443 | 508 |
| **F4** | 10208.50 | 444 | 513 |

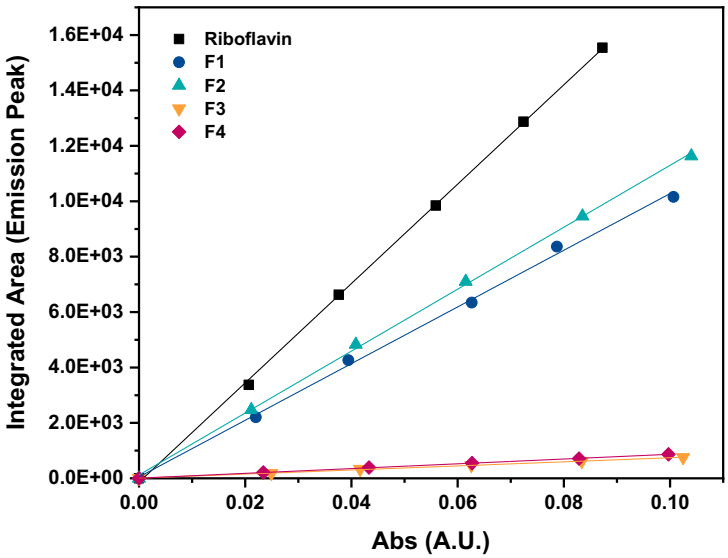



**Figure S2:** Plot of integrated emission peak against absorbance value of flavins **F1-4** and reference compound riboflavin in DMSO.

## 5 Singlet Oxygen Quantum Yield Calculations

The singlet oxygen quantum yield ($\Phi_\Delta$) of flavins **F1-4** were calculated using the singlet oxygen probe, 1,3-diphenylisobenzofuran (DPBF, 97%, Acros Organics) according to the guidelines published by Bresolí-Obach *et al*.> The light source used was a 450 nm LED (18 W, HepatoChem, US) with the light output attenuated using a 2" x 2" Absorptive Neutral Density Filter (OD 3.0, Thor Labs, UK) as to avoid considerable self-bleaching of DPBF (blank control). $Ru(bpy)_3^{2+}$ (dichloro hexahydrate, 99.95%, Sigma-Aldrich, UK) was chosen as the reference $\phi_\Delta$ for calculations ($\Phi_\Delta$ = 0.57 ± 0.06 in MeCN)[6] according to equation (2):

$$\Phi_{\Delta;\mathrm{Sample}} = \Phi_{\Delta;\mathrm{Ref}} \frac{(Slope_{Sample} - Slope_{Control}) \cdot (1 - 10^{-A_{Ref}})}{(Slope_{Ref} - Slope_{Control}) \cdot (1 - 10^{-A_{Sample}})} \left( \frac{\eta^2_{Sample}}{\eta^2_{Ref}} \right) \quad (2)$$

Where Slope = rate of DPBF photobleaching, Sample = flavin **F1**-**4**, Control = DPBF alone, Ref = $Ru(bpy)_3^{2+}$, A = absorbance value at irradiation wavelength (450 nm) and η = refractive index of the solvent.

**General procedure:** DPBF (50 µM) was irradiated with stirring at 450 nm in the presence of flavin (20 µM) or $Ru(bpy)_3^{2+}$ (15 µM) in MeCN (3 mL) at RT with the reaction vessel open to air. Aliquots of the reaction (100 µL) were taken every 30 s for 3 min and measured by UV-Vis spectroscopy. The decrease of the $\lambda_{max}$ of DBPF (410 nm) was then evaluated and plot according to $A/A_0$ vs. time as shown below (**Figure S2**). Experiments were carried out three separate times using fresh stock solutions of the reagents. The experimental error was estimated by combining the $\Phi_{\Delta;Ref}$ error and the standard deviation of the average $\Phi_\Delta$ for the flavin samples.

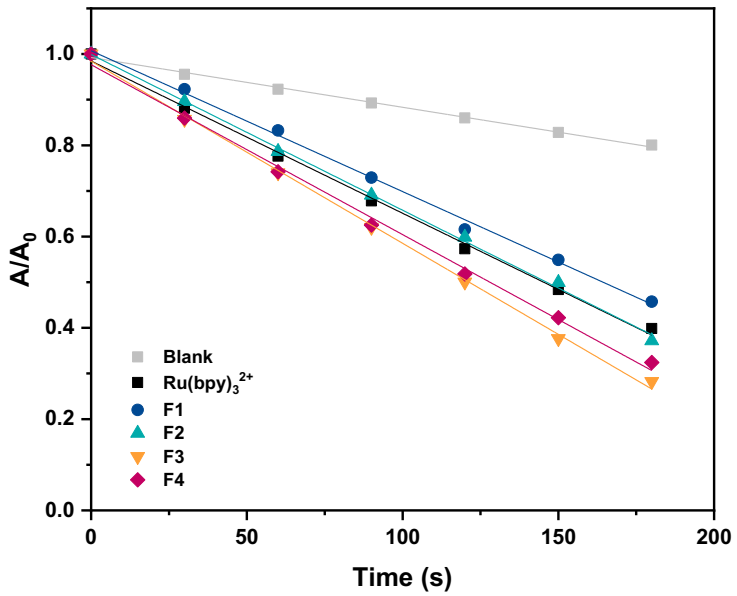



**Figure S3:** Plot of DPBF bleaching in the presence of flavins **F1-4** or reference compound $Ru(bpy)_3^{2+}$ under 450 nm irradiation in MeCN. Blank = DPBF alone with no photosensitiser.

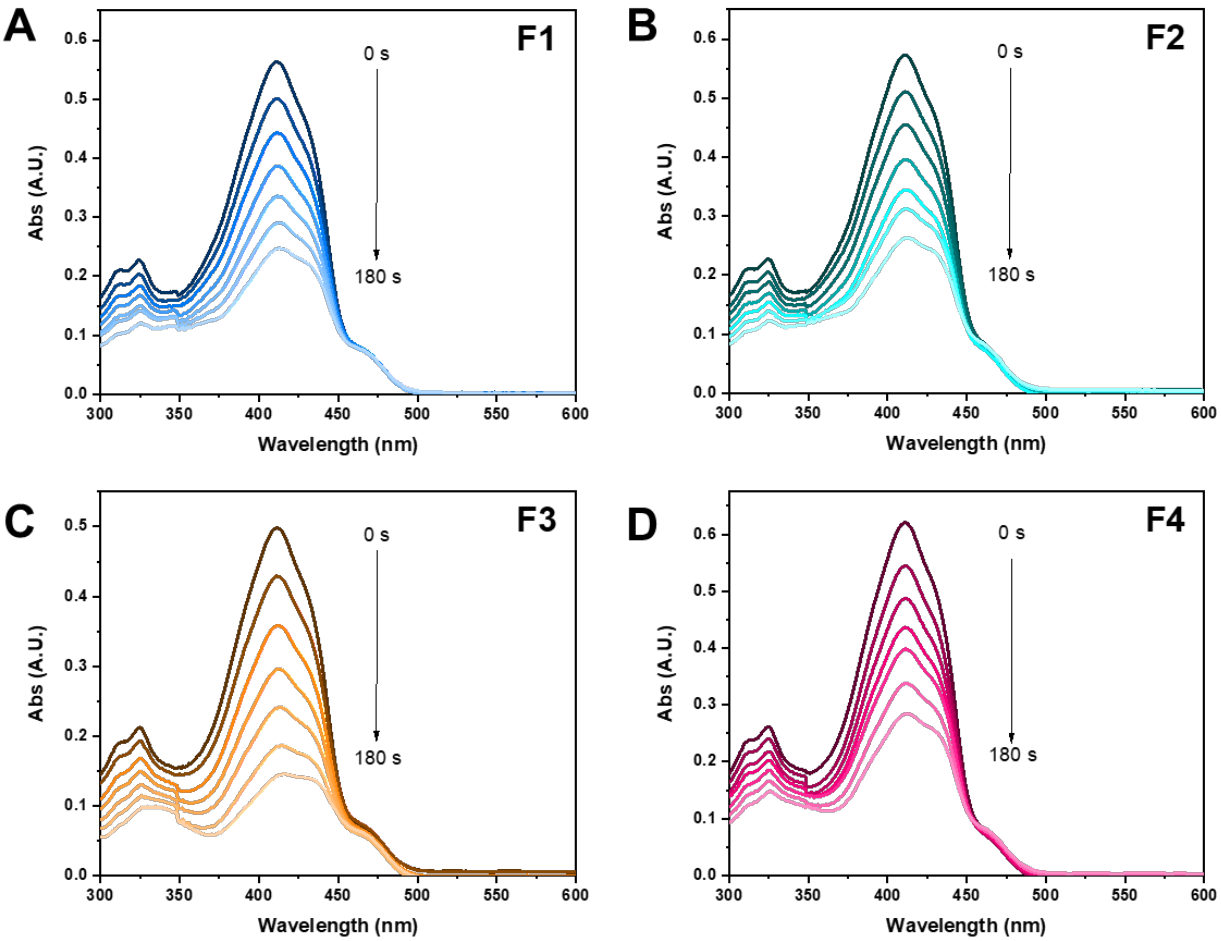



**Figure S4:** UV-Vis absorption spectra of DPBF bleaching in the presence of flavins **F1-4** under 450 nm irradiation in MeCN.

## 6 Flavin Photostability

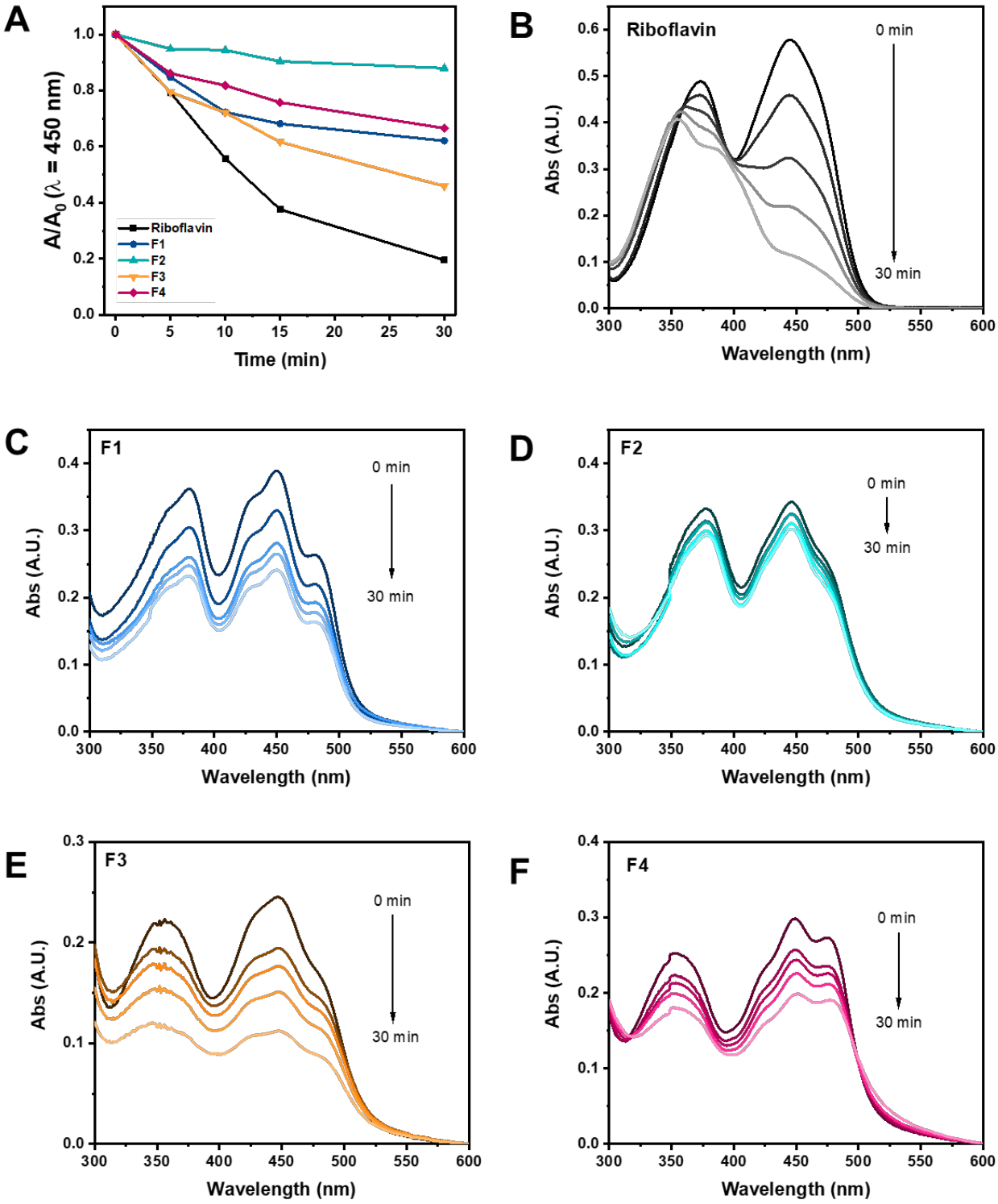



**Figure S4**: (A) Flavin photostability (100 µM) in PBS (0.1% DMSO) irradiated with $1x10^5$ lx (35 mW/cm$^2$) where A = Abs at 450 nm and $A_0$ = Abs at 450 nm at 0 min. (B-F) Associated UV-Vis spectra of riboflavin and **F1-4** photostability. Experiments were performed using a quartz cuvette and measured using an Agilent Cary 300 Spectrophotometer.

## 7 Bacterial Culture and Phototoxicity Assay

An adapted method reported by Maisch *et al.* was used to evaluate the phototoxicity of flavin compounds towards *E. coli* BL21(DE3).>

After an overnight aerobic culture in LB broth at 37 °C with shaking at 225 rpm, the resulting *E. coli* were centrifuged at 2,500 rpm for 10 minutes. The pellet was resuspended in PBS (Sigma, 1x, pH 7.4) to an $OD_{600}$ value of 0.6 (~$1\times10^8$ bacteria per ml). Bacterial solutions were then combined with varying concentrations of **F1-4** and riboflavin (0, 1, 10, 100 μM) dissolved in PBS in a 1:1 ratio and left to incubate for 20 minutes in dark. Aliquots of negative controls (0.5% DMSO in PBS; PBS only) and 100 μM flavin-bacteria samples were covered in foil to remain in dark, while all samples were plated onto a 96-well plate and exposed to white LED irradiation ($1x10^5$ lx) for 15 minutes. After 15 minutes of irradiation or darkness, each sample was serially diluted to its respective, appropriate dilution and plated onto an LB agar plate with 50 μg/mL kanamycin. Plates were incubated overnight at 37 °C in the dark. Survival of bacteria was determined by counting colony forming units (CFUs) the next day.

For time point data, aliquots of 100 μM flavin-bacteria solutions were taken at 5-minute increments of irradiation (0, 5, 10, and 15 minutes) and evaluated similarly as above. Time point experiment with one-minute increments, between 0 and 5 minutes, were conducted for 100 μM **F4**-bacteria samples.

All data was generated with technical replicates and biological triplicates.

All statistical analysis was done with Graphpad Prism 9. One-way ANOVA with Tukey's multiple comparisons test, was performed to evaluate the impact of flavin concentration compared to the control (PBS). Two-way ANOVA with Bonferroni's multiple comparison test, was performed to evaluate the impact of light given the same flavin concentration with different irradiation conditions (dark and light). Significance levels are defined as the following: ns for $p>0.05$, * for $p\leq0.05$, ** for $p\leq0.01$, *** for $p<0.001$, and **** for $p<0.0001$.

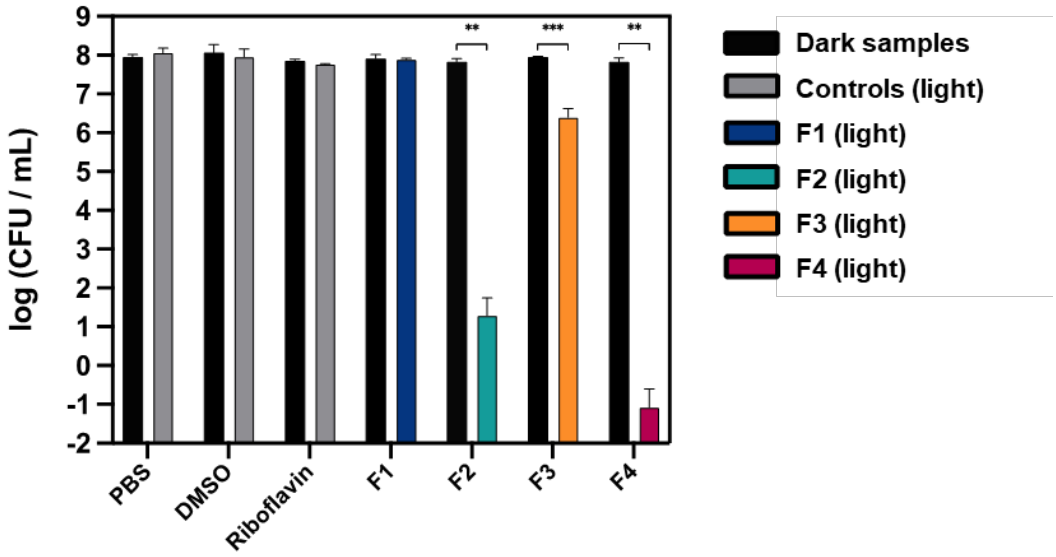



**Figure S5:** Photodynamic inactivation of *E. coli* incubated with 100 µM of flavin compound irradiated with white LED light (1x10$^5$ lx, 35 mW/cm$^2$) or incubated in the dark for 15 min in PBS (0.5% DMSO). Data are expressed as the mean ± SD of three biological replicates. Significance levels are defined as the following: ns for $p>0.05$, * for $p\leq0.05$, ** for $p\leq0.01$, *** for $p<0.001$, and **** for $p<0.0001$.

### *7.1 E. coli Log$_{10}$ Reductions*

**Table S1:** Table of *E. coli* $\log_{10}$ reductions in the presence of flavin under irradiation.

| Flavin | Irradiation time (min) | Concentration (μM) | $\log_{10}$ reduction[a] |
|---|---|---|---|
| **Riboflavin** | 15 | 1 | 0.2 |
| | 15 | 10 | 0.2 |
| | 5 | 100 | 0.0 |
| | 10 | 100 | 0.0 |
| | 15 | 100 | 0.0 |
| **F1** | 15 | 1 | 0.2 |
| | 15 | 10 | 0.2 |
| | 5 | 100 | 0.1 |
| | 10 | 100 | 0.1 |
| | 15 | 100 | 0.1 |
| **F2** | 15 | 1 | 0.2 |
| | 15 | 10 | 0.8 |
| | 5 | 100 | 3.4 |
| | 10 | 100 | 5.9 |
| | 15 | 100 | 7.0 |
| **F3** | 15 | 1 | 0.2 |
| | 15 | 10 | 0.4 |
| | 5 | 100 | 1.3 |
| | 10 | 100 | 1.7 |
| | 15 | 100 | 1.8 |
| **F4** | 15 | 1 | 0.2 |
| | 15 | 10 | 2.8 |
| | 1 | 100 | 4.1[b] |
| | 2 | 100 | 5.8[b] |
| | 3 | 100 | 6.4[b] |
| | 4 | 100 | 7.6[b] |
| | 5 | 100 | 8.4 |
| | 10 | 100 | 8.7 |
| | 15 | 100 | 9.1 |

[a] calculated by $\log_{10}$(control) - $\log_{10}$(sample) at respective irradiation time

[b] calculated by $\log_{10}(t_0)$ - $\log_{10}(t_{sample})$ where $t_0 = 7.9$

### *7.2 SIM Imaging*

Structured illumination microscopy (SIM) of *E. coli* BL21(DE3) was performed using a culture of *E. coli* BL21(DE3) grown overnight at an $OD_{600}$ of ~1. Prior to SIM imaging the cultures were centrifuged at 3000 rpm for 5 minutes and resuspended in PBS (1x. pH 7.4) to an $OD_{600}$ of around 0.5. The *E. coli* suspension was incubated for 1 hour with 100 µM of each one of the flavins (riboflavin, **F1** or **F2** in PBS). The bacteria were then centrifuged at the same settings and the bacterial pellet resuspended in PBS (1x. pH 7.4) to remove excess flavin. 2 µl of the sample were deposited on a glass coverslip and an agarose pad was positioned over the sample to prevent the bacteria from moving during imaging. Another coverslip was positioned on top to minimise drying of the agarose pads.

Images of the sample were collected using 3-color SIM for optical sectioning. A ×60/1.2 NA water immersion lens (UPLSAPO 60XW, Olympus) focused the structured illumination pattern onto the sample, and the same lens was also used to capture the fluorescence emission light before imaging onto an sCMOS camera (C11440, Hamamatsu). The wavelength used for excitation was 488 nm (iBEAM-SMART-488, Toptica). Images were acquired using custom SIM software.

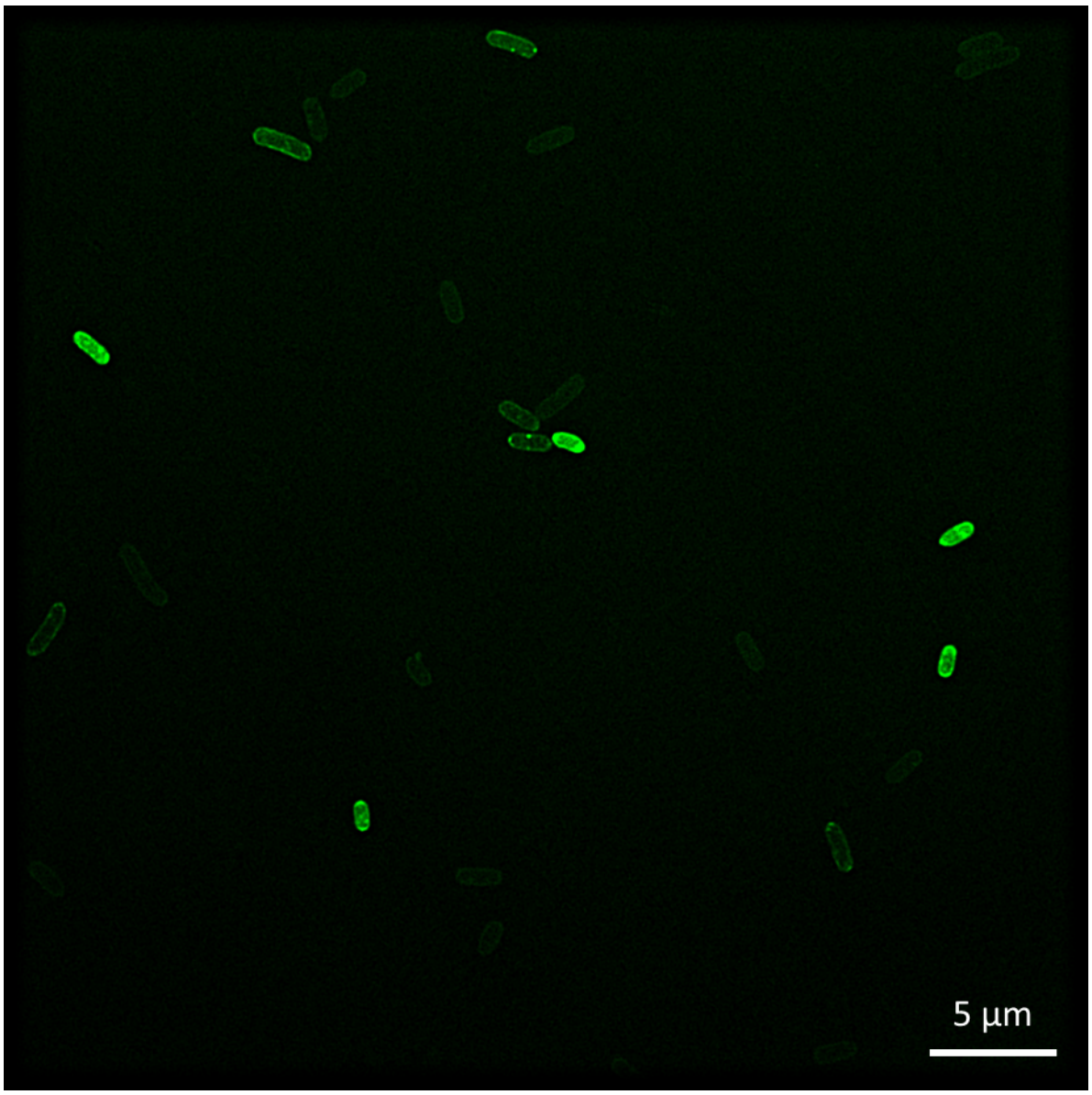



**Figure S6:** Large field of view (42.8 x 42.8 µm) of *E. coli* after incubation with **F2** (100 µM).

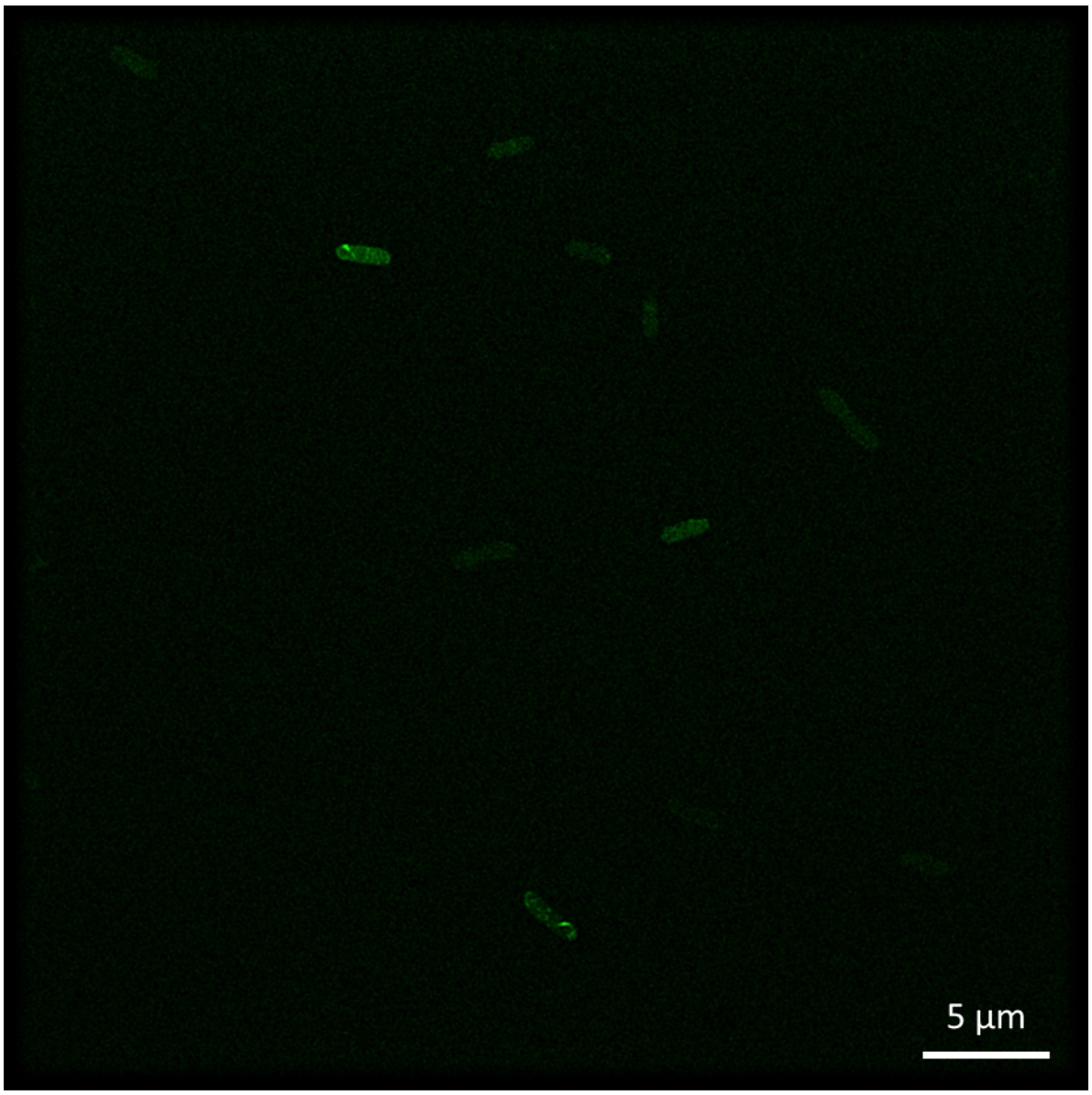



**Figure S7:** Large field of view (42.8 x 42.8 µm) of *E. coli* after incubation with **F1** (100 µM).

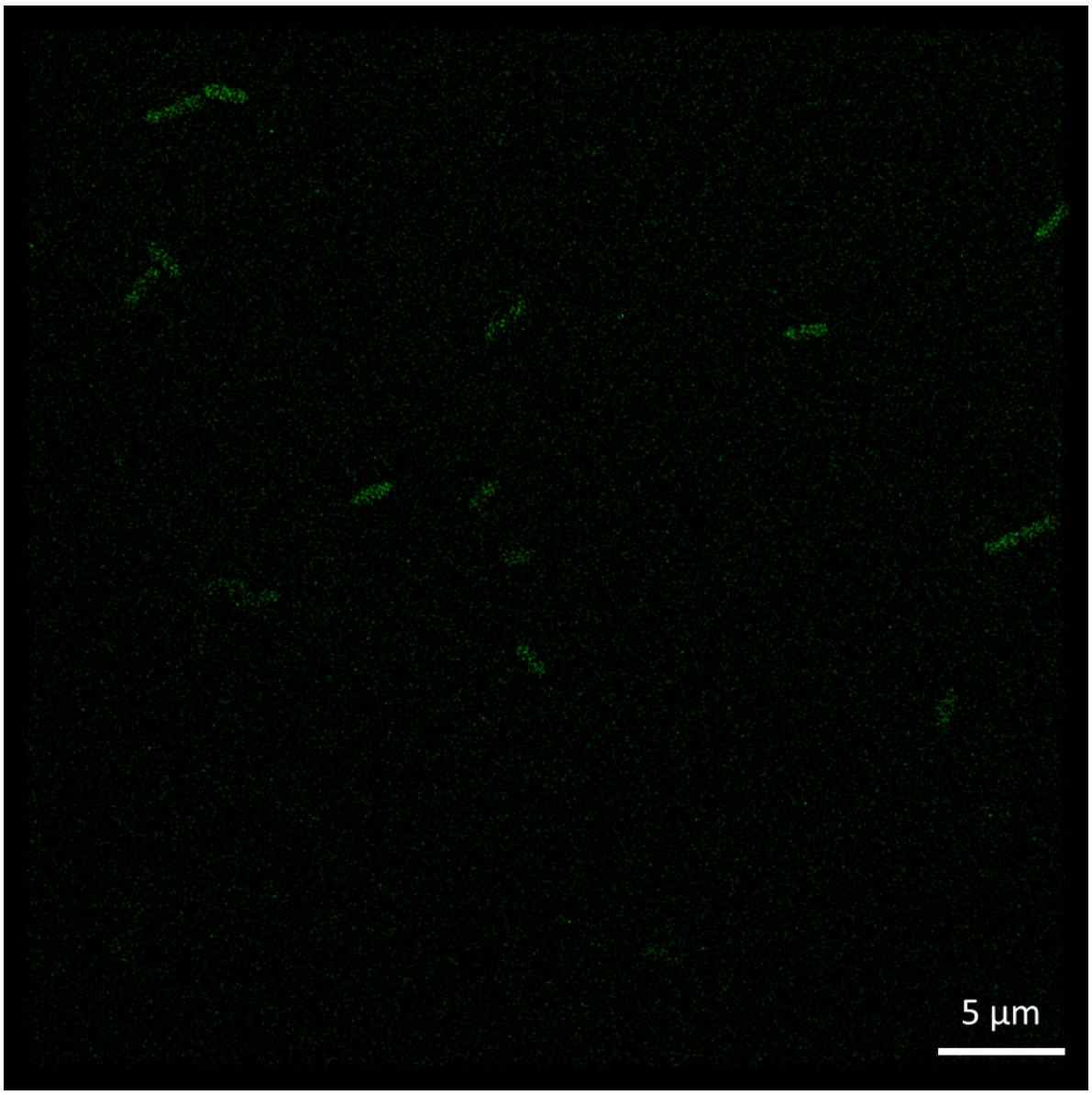



**Figure S8:** Large field of view (42.8 x 42.8 µm) of *E. coli* after incubation with riboflavin (100 µM).

# 8 Virology and $TCID_{50}$ Assay

### *8.1 Cell Culture*

17Cl-1 murine fibroblasts cells and murine hepatitis virus A59 strain (MHV-A59) were kindly gifted by Professor Ian Goodfellow's lab (Department of Pathology, University of Cambridge, UK). 17Cl-1 cells were resurrected from cold-storage cell banks and propagated using routine cell culture protocols in complete growth medium (Dulbecco's modified Eagle's medium low glucose 1 g/L (DMEM, Life Technologies) supplemented with 5% foetal bovine serum (Merck), 6% tryptose phosphate broth (Merck), 1x non-essential amino acids (Gibco), 1x antibiotic-antimycotic (Thermo Fisher Scientific) and 1x L-Glutamine (Gibco). 17Cl-1 cells were seeded (100 µl per well) into 96-well plates (untreated, flat bottom) at $1 \times 10^4$ cells/well at 37°C for 24 hours in 5% $CO_2$ prior to the day of testing.

### *8.2 Virus Propagation*

MHV-A59 was propagated in 17Cl-1 cells at a multiplicity of infection (MOI) of 0.01 $TCID_{50}$ per cell. MHV-A59 was clarified by centrifugation at 3000 rpm for 10 min and stored in aliquots at -80°C. The $TCID_{50}$ was calculated using the Reed and Muench method.[1]>

### *8.3 Flavin Treatment of MHV-A59*

The efficacy of the flavins were enumerated utilizing modified methods from ISO 18184 and Leibowitz *et al.*[1]> Flavins **F1-4** and positive control, Riboflavin, were solubilized in DMSO. 100 µM working stock solutions were then created by diluting each DMSO solution into PBS. To control for the effect of DMSO on MHV-A59, DMSO was diluted into PBS at the same concentration. A white light apparatus was set up to cover a 4 x 5 (row x column) area of a 96-well plate with the same level of light intensity ($1x10^5$ lx). The stock solutions were then diluted in replicates of four to a total of 180 µl in PBS at 1, 5, and 10 µM concentrations for each sample. Virus stocks of MHV-A59 were thawed on ice prior to use. 20 µl of MHV-A59 stock (1.4 x 109 PFU/ml) was then mixed into each well, and the 4 x 5 well matrix was treated with the light or incubated in the dark at room temperature for a specified time (5, 10, or 15 minutes).

### *8.4 Virus Recovery and $TCID_{50}$ Assay MHV-A59*

After treatment, the samples were mixed into 320 µl of Gibco Difco™ Beef Extract (1.5% w/v beef extract in $ddH_2O$; Life Technologies) and rolled for 15 minutes to chelate any free ions in the solution. The samples then were serially diluted ($10^0$ - $10^{-7}$) in infectivity media (Dulbecco's modified Eagle's medium low glucose 1 g/L (DMEM, Life Technologies) supplemented with 2.5% fetal bovine serum (Merck), 3% tryptose phosphate broth (Merck), 1x non-essential amino acids (Gibco), 1x antibiotic-antimycotic (Thermo Fisher Scientific) and 1x L-Glutamine (Gibco]) and added in triplicate to 17Cl-1 cell plates. For each sample or control there were 4 replicate treatments (in light or dark); each treatment replicate was plated in triplicate. Infected 17Cl-1 plates were

incubated at 37°C in a 5% $CO_2$ atmosphere for 24 hours. Plates were scored for cytopathic effect (CPE) by microscopy and viral titres were determined by the Reed and Muench 50% tissue culture infectious dose ($TCID_{50}$) end point method.[1]> Outliers were removed from the four replicates using an outlier test. The full protocol was repeated in triplicate on different days to account for any variability in the assay.

### *8.5 MHV-A59 $Log_{10}$ Reductions*

**Table S2:** Table of MHV-A59 $log_{10}$ reductions in the presence of flavin under irradiation.

| Flavin | Irradiation time (min) | Concentration (μM) | $Log_{10}$ reduction[a] |
|---|---|---|---|
| **Riboflavin** | 10 | 1 | 1.2 |
| | 10 | 5 | 3.2 |
| | 5 | 10 | 2.7 |
| | 10 | 10 | 3.4 |
| | 15 | 10 | 3.7 |
| **F1** | 10 | 1 | 0.0 |
| | 10 | 5 | 1.7 |
| | 5 | 10 | 1.2 |
| | 10 | 10 | 2.1 |
| | 15 | 10 | 2.2 |
| **F2** | 10 | 1 | 2.1 |
| | 10 | 5 | 3.9 |
| | 5 | 10 | 3.0 |
| | 10 | 10 | 3.8 |
| | 15 | 10 | 5.0 |
| **F3** | 10 | 1 | 2.1 |
| | 10 | 5 | 4.1 |
| | 5 | 10 | 3.9 |
| | 10 | 10 | 4.8 |
| | 15 | 10 | 5.4 |
| **F4** | 10 | 1 | 3.4 |
| | 10 | 5 | 4.6 |
| | 5 | 10 | 3.8 |
| | 10 | 10 | 5.9 |
| | 15 | 10 | 6.7 |

[a] calculated by $log_{10}$(control) - $log_{10}$(sample) at respective irradiation time

# 9 Viability and Phototoxicity Assay

## *9.1 Cell Culture*

Normal human lung fibroblasts, WI-38, were purchased from American Type Culture Collection (ATCC) and murine fibroblast cell line, 17Cl-1, was kindly provided by Ian Goodfellow's lab (Department of Pathology, University of Cambridge, UK). WI-38 cells were cultured in Dubelco's modified essential medium (Gibco) with 10% fetal bovine serum (FBS, Gibco) containing penicillin (50 IU/mL) and streptomycin (50 µg/mL) (Thermo Fisher Scientific). 17Cl-1 cells were cultured as described in **Section 8.1**. All Cell lines were maintained in a humidified environment at 37°C with 5% $CO_2$ and routinely tested to confirm the absence of Mycoplasma. All *in vitro* experiments were conducted between 60% and 80% confluent cultures at passage 6–15 (WI-38) and 25–35 (17Cl-1).

## *9.2 MTS viability assay*

The effect on cell viability of WI-38 and 17Cl-1 cells after treatment with **F1**–**F4** and riboflavin was determined using the commercially available MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H* tetrazolium] assay (Promega). The MTS tetrazolium compound is reduced by cells into a coloured formazan product which is soluble in cell culture media. It can be detected colorimetrically between 450-540 nm with the measured absorbance directly proportional to the amount of metabolically active cells in culture. Cells were seeded into clear 96-well plates containing 10000 cells/well in 100 µL complete growth medium and cultured for 24 hours at 37 °C and 5 % $CO_2$. Subsequently, cells were treated with varying concentrations of **F1**–**F4** and riboflavin (0.01–100 µM) dissolved in complete growth media containing 0.1% DMSO. After further 24 hours incubation at 37 °C and 5 % $CO_2$, 20 µL of CelTiter 96® $AQ_{ueous}$ One Solution (Promega) was added into each well and incubated at 37°C, 5% $CO_2$ for 1–4 hours, according to the manufacturer's instruction. The absorbance of each well was measured at 490 nm using a plate reader (Spark, Tecan). Control measurements included negative control of cells with DMEM, cells with DMEM containing 0.1% DMSO, cell-free culture media (blank) and cell-free sample dilutions in culture media to evaluate potential sample interferences with MTS assay. All experiments were conducted in biological triplicates. The percentage cell viability was calculated according to equation (3):

$$Cell\ viability\ (\%) = 100\ x \left( \frac{Absorbance\ of\ treated\ cells - Absorbance\ of\ blank}{Absorbance\ of\ control - Absorbance\ of\ blank} \right) \quad (3)$$

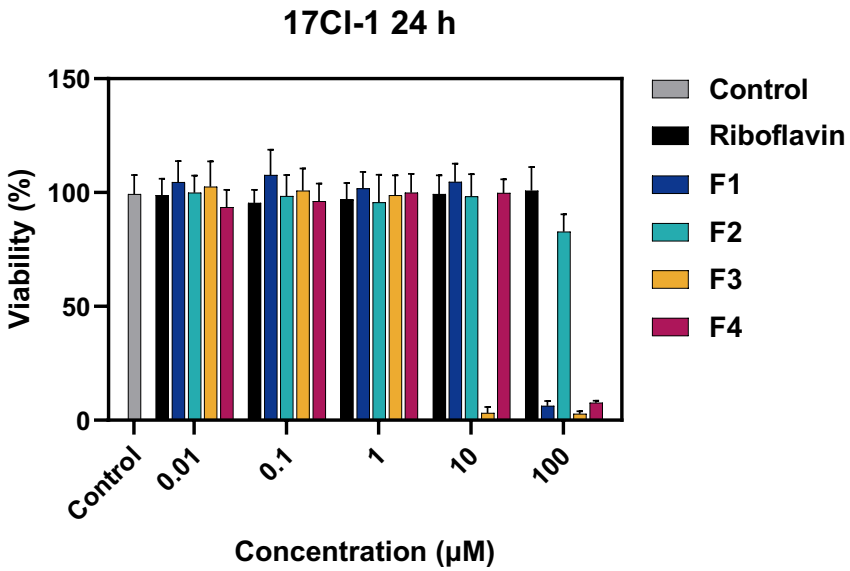
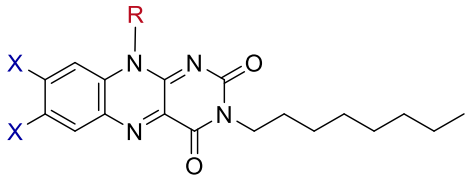
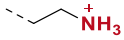
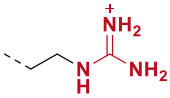
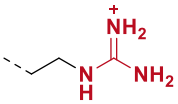



**Figure S9**. *In vitro* cytotoxicity effect of **F1**–**F4** and riboflavin on 17Cl-1 cells after 24 h incubation determined by MTS assay. Data are expressed as the mean ± SD of three biological replicates.

**Table S3:** $IC_{50}$ and cLogP values for flavins **F1-4** and riboflavin.




| Flavin | X | R | $IC_{50}$ (µM)[a] | | cLogP[b] |
|---|---|---|---|---|---|
| | | | WI-38 | 17Cl-1 | |
| **Riboflavin** | Me | Ribityl chain | >100 | >100 | -2.07 |
| **F1** | Me |  | 96.1 | 70.2 | -0.55 |
| **F2** | Me |  | >100 | >100 | 2.59 |
| **F3** | Br |  | 30.9 | 3.66 | 0.22 |
| **F4** | Br |  | >100 | 50.9 | 3.36 |

[a] determined after 24 h incubation by MTS assay
[b] determined using DataWarrior V5.5.0

### *9.3 Phototoxicity assay on WI-38 human cells*

An adapted method reported by Maisch *et al.* was used to conduct phototoxicity studies on human lung fibroblasts, WI-38.> Cells were seeded into 96-well plates containing 10000 cells/well in 100 µL complete growth medium and cultured for 24 hours at 37 °C and 5 % $CO_2$. On the next day, the growth media was removed, and cells were treated with 10 µM **F1**–**F4** and riboflavin dissolved in DMEM without serum and phenol red (Gibco) containing 0.1% DMSO. The resulting mixtures were then incubated in the dark for 5 min and then either illuminated with $1x10^5$ lx for 5, 10 or 15 min, or incubated further in the dark for 15 min (dark control). After irradiation, the flavin solutions were removed and 100 µL of DMEM with 10% FBS and without phenol red was added to each well and incubated over night at 37 °C and 5 % $CO_2$. Subsequently, 20 µL of MTS reagent was added into each well and incubated at 37°C and 5% $CO_2$ for 1–4 hours and the absorbance of each well was measured at 490 nm using a plate reader. Control measurements included negative control untreated cells with light and in the dark containing 0.1% DMSO and cells treated with flavins for 15 min in the dark. All experiments were conducted in biological triplicates. The percentage cell viability was calculated according to equation (3).

## 10 Nucleic acid photocleavage

**General procedure:** Riboflavin (**Rbf**) or flavin **F1-4** (10 µM from DMSO stock solutions) were mixed with either pUC18 DNA (Thermo Scientific, 0.50 µg) or EGFP-encoding mRNA (~1k nt) as the ssRNA model (TriLink Biotech, 0.88 µg) dissolved in PBS (40 µL) to give a final DMSO concentration of 0.1%. A 20 µL aliquot was taken after 15 min of white LED irradiation ($1x10^5$ lx). Prior to loading, 2 µL of 6X Orange-G loading dye in glycerol were added to the aliquot and mixed vigorously. The gel was electrophoresed at 80 V for 35 min with 1 % agarose gels in 1X Tris-acetate-EDTA (TAE) buffer. Gels were then imaged in a Syngene G:BOX Gel Documentation System and band % quantification was performed using ImageJ Gel Analyzer.

# 11 References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