Electronic Supplementary Information

Model systems for flavoenzyme activity: an investigation of the role functionality attached to the C(7) position of the flavin unit has on redox and molecular recognition properties.

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General experimental:

Melting points are uncorrected. ^{1}H NMR spectra were recorded on Bruker AC 200 MHz or Bruker AC 400 MHz spectrometers. All NMR spectra were recorded in the presence of tetramethylsilane (TMS) as a reference (δ = 0.00 ppm). MS-spectra were recorded at the EPSRC National Mass Spectrometry Service Centre, Chemistry Department, University of Wales, Swansea.

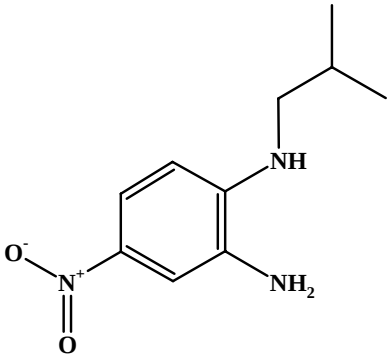
All electrochemical experiments were performed using a CH Instruments 440A electrochemical workstation. The electrolyte solution (0.1 M) was prepared from recrystallized Bu_4NPF_6 and dry dichloromethane. A platinum disk working electrode, a platinum wire counter electrode and a silver wire reference electrode were used in all electrochemical measurements. Ferrocene (~1 x 10^{-5} M) was added to the electrolyte solution as an internal reference. The ferrocene/ferrocenium redox couple was adjusted to zero volts, and all electrochemical data reported here are referenced to this redox couple. The solution was purged for two minutes with nitrogen prior to recording electrochemical data and the voltammograms were recorded under an atmosphere of nitrogen.

Transmission IR spectra were recorded as thin films or KBr disks using a Perkin-Elmer RX FT-IR system (4 scans with 4 cm^{-1} resolution) or as KBr discs or using a "golden gate" system using a JASCO FT-IR 410 spectrometer (16 scans with 4 cm^{-1} resolution).

The solvents used to conduct syntheses were dried before the experiments using standard methods.

<u>Syntheses of flavin derivatives</u>:
Synthesis of 7-Nitroflavin (6).

*N^1-Isobutyl-4-nitrobenzene-1,2-diamine (**8**)*

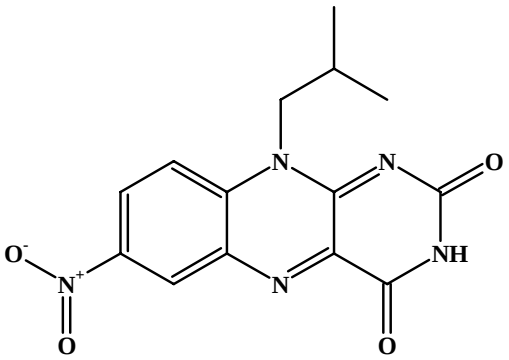


2-Chloro-5-nitroaniline (15 g, 87 mmol), isobutylamine (21 mL) and potassium carbonate (20 g) were heated under reflux in DMF for 3 days. The reaction was monitored by TLC (petroleum ether 40-60 /ethyl acetate mixture 4/1 (v/v)). When no more product was formed, the solvent was removed *in vacuo* and the product was purified by column chromatography on silica gel with acetone/DCM (15/85 (v/v)) mixture as an eluent, which afforded dark red residue (0.50 g, mixture of the product and starting material in 7:3 ratio, 2 % yield) that was used in the following reactions without further purification.

For analytical purposes product **8** was purified further by repeated chromatography with acetone/DCM (5/95 v/v) as an eluent, which afforded **8** as a dark red viscous liquid.

^{1}H NMR (200 MHz, $CDCl_3$): δ 7.77 (1H, dd, J_1=8.8, J_2=1.2); 7.58 (1H, d, J=1.2); 6.47 (1H, d, J=8.8); 4.40 (1H, br.s); 3.34 (2H, br.signal); 2.98 (2H, m); 1.90 (1H, m); 0.98 (6H, d, J=8.0). ^{13}C NMR (100 MHz, DMSO-d_6): δ 145.37; 137.93; 131.67; 119.60; 112.79; 108.14; 51.30; 28.05; 20.47. IR (golden gate, ν, cm^{-1}): 3391.2; 3352.6; 2950.5; 2870.5; 1590.0; 1536.0; 1467.6; 1431.9; 1247.7; 1162.9; 1088.6; 949.8; 885.2; 746.3; 645.1. MS m/z (Accurate mass measurement, ES+) 210.1237 $[M+H]^+$ (210.1238 calc.).

*7-Nitro flavin (**6**)*

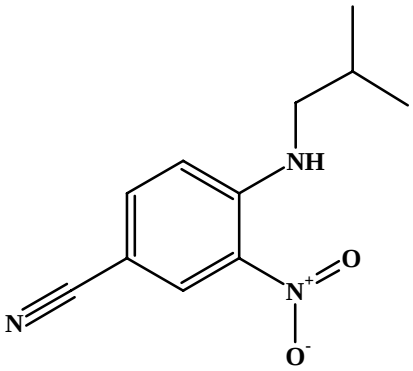


Compound **8** (0.50 g, 2.4 mmol), alloxan monohydrate (0.4 g) and boron oxide (0.34 g) were dissolved in glacial acetic acid (20 mL). The reaction mixture was stirred at 90 ^{0}C for 2 h, and the solvent was removed *in vacuo*. The product was purified by column chromatography on silica gel with acetone/DCM (1/4 (v/v)) as an eluent followed by crystallization from ethanol, which afforded product **6** as yellow solid (0.12 g, 17 % yield).

M.p. 270 °C (dec). ^{1}H NMR (200 MHz, $CDCl_3$ with 3 drops of DMSO-d_6): δ 11.30 (1H, s); 8.48 (1H, d, J=0.8); 8.06 (1H, dd, J_1=8.8, J_2=0.8); 7.40 (1H, d, J=8.8); 4.06 (2H, br. signal); 1.87 (1H, sept, J=8.0); 0.50 (6H, d, J=8.0). ^{13}C NMR (100 MHz, DMSO- d_6): δ. 158.85; 155.27; 150.96; 143.88; 140.12; 136.82; 133.63; 128.00; 127.41; 117.40; 50.99; 26.88; 19.51. IR (golden gate, ν, cm^{-1}): 3031.6; 2965.0; 2832.9; 1712.5; 1655.6; 1560.1; 1509.0; 1481.1; 1403.9; 1342.2; 1243.9; 1205.3; 1171.5; 1081.9; 1039.4; 832.1; 742.5; 645.1. MS m/z (Accurate mass measurement, ES+) 316.1040 $[M+H]^+$ (316.1042 calc.).

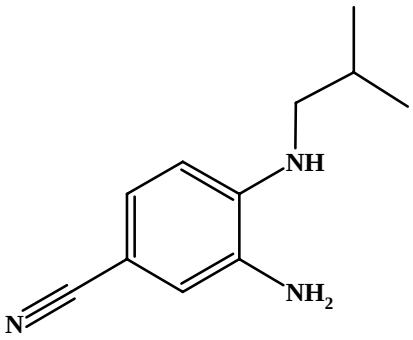
Synthesis of 7-Cyano flavin (5)

*4-(Isobutylamino)-3-nitrobenzonitrile (**9**)*

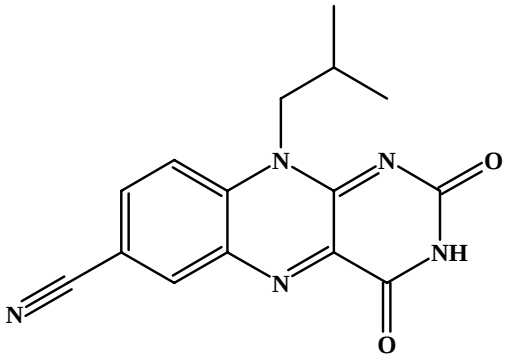


4-Chloro-3-nitrobenzonitrile (10 g, 54.7 mmol), isobutylamine (12 mL) and triethylamine (12 mL) were dissolved in THF (125 mL). The reaction mixture was stirred at room temperature for 4 h. The solvent was removed *in vacuo*, and the obtained residue was dissolved in DCM and washed with water (2 x 100 mL). The organic layer was separated and dried over $MgSO_4$, filtered and the solvent was removed *in vacuo*. The product was purified by column chromatography on silica gel with petroleum ether 40-60 as an eluent, affording **9** as a yellow solid (9.55 g, 85 %). M.p. 71-73 ºC. ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.46 (2H, s); 7.52 (1H, dd, J_1=8.0, J_2=0.9); 6.84 (1H, d, J=8.0); 3.13 (2H, m); 2.0 (1H, sept, J=8.0); 0.98 (6H, d, J=8.0). ^{13}C NMR (100 MHz, $CDCl_3$): δ. 147.39; 137.57; 132.28; 131.27; 118.04; 97.84; 50.80; 27.93; 20.97. IR (JASCO, golden gate, ν, cm^{-1}): 3368.1; 3377.8; 2951.5; 2870.5; 2220.6; 1627.6; 1560.1; 1529.3; 1461.8; 1406.8; 1365.4; 1275.7; 1241.9; 1160.9; 1057.8; 920.8; 820.6; 762.7. MS m/z (Accurate mass measurement, ES+) 219.1002 $[M+H]^+$ (219.1002 calc.).

*3-Amino-4-(isobutylamino)benzonitrile (**10**)*



Compound **9** (9.55 g, 46.5 mmol) was dissolved in methanol (550 mL). Palladium on carbon (10%, 1.5 g) and ammonium formate (23 g) were added, and mixture was stirred at RT overnight. The palladium on carbon was removed by filtration, and the methanol was removed *in vacuo*. The obtained residue suspended in DCM. Non-soluble ammonium formate was filtered off and washed with acetone. The filtrate was collected and dried *in vacuo* to give 16 g of brown residue. Due to the low stability of the product **10**, no further purification was performed.



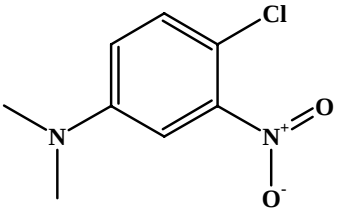
7-Cyano flavin (5)

Compound **10** (16 g, considering that there is not more than 46.5 mmol of the diamine), alloxan monohydrate (9.2 g) and boron oxide (7.7 g) were dissolved in glacial acetic acid (460 mL). Reaction mixture was stirred at 90 °C for 3 h, and the solvent was removed *in vacuo*. The product was deposited on silica and purified by column chromatography on silica gel with acetone/DCM (2/8 (v/v)) as an eluent followed by crystallization from ethanol to afford **5** as a yellow solid (7.46 g, 54 %).
M.p. 260 ºC (dec). ^{1}H NMR (200 MHz, DMSO-d_6): δ 11.40 (1H, br.s); 8.56 (1H, d, J=0.6); 8.09 (1H, dd, J_1=8.8, J_2=0.6); 7.97 (1H, d, J=8.8); 4.30 (2H, br. d); 2.18 (1H, m); 0.83 (6H, d, J=8.0). ^{13}C (100 MHz, DMSO-d_6): δ 159.70; 155.88; 151.86; 141.06; 136.77; 136.32; 134.35; 131.27; 118.73; 118.01; 108.28; 50.85; 27.09; 20.07.

IR (golden gate, ν, cm^{-1}): 3191.6; 3064.3; 2234.1; 1717.3; 1667.2; 1619.0; 1584.2; 1551.5; 1520.6; 1398.1; 1241.0; 1213.0; 1184.1; 1105.0; 930.5; 834.1; 773.3; 691.4. MS m/z (Accurate mass measurement, ES+) 296.1142 $[M+H]^+$ (296.1147 calc.).

Synthesis of 7-Dimethylamino flavin (1).

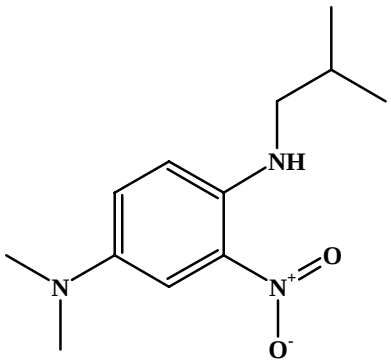
*N-(4-Chloro-3-nitrophenyl)-N,N-dimethylamine (**11**)*



4-Chloro-3-nitroaniline (5.18 g, 30.0 mmol) and potassium carbonate (10.45 g, 76.5 mmol) were suspended in water (30 mL) at 100 ºC. Dimethyl sulphate (6.0 mL, 8.0 g, 63.4 mmol) was added to the reaction mixture *via* dropping funnel over 2 h. The mixture was heated under reflux for 3 days, and every day the same portions of potassium carbonate and dimethyl sulphate were added. The reaction was then cooled to room temperature and the product was extracted from water by DCM. The organic phase was collected, dried and filtered and the solvent was removed *in vacuo*. The product was purified by column chromatography on silica gel with DCM/petroleum ether 40-60 (1/1 (v/v)) as an eluent, affording **11** as orange-yellow solid (2.80 g, 47 %).

M.p. 62-64 ºC. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.35 (1H, d, J=6.0); 7.11 (1H, d, J=3.2); 6.79 (1H, dd, J_1=8.8, J_2=2.8); 3.03 (6H, s). ^{13}C NMR (100 MHz, $CDCl_3$): δ 149.18; 131.85; 116.28; 112.85; 106.03; 40.32. IR (golden gate, ν, cm^{-1}): 3094.2; 2887.9; 2811.7; 1608.3; 1605.5; 1504.2; 1360.5; 1232.3; 1178.3; 1116.4; 1064.8; 881.3; 835.0; 805.1; 783.9; 749.2; 685.6. MS m/z (Accurate mass measurement, ES+) 201.0425 $[M+H]^+$ (201.0425 calc.).

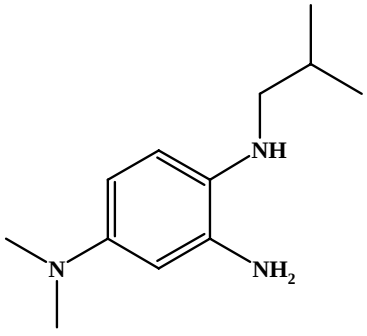
*N1-Isobutyl-N4,N4-dimethyl-2-nitrobenzene-1,4-diamine (**12**)*



Compound **11** (2.70 g, 13.5 mmol), isobutylamine (3 mL) and potassium carbonate (5.53 g) were stirred in anhydrous DMF (40 mL). The mixture was heated under reflux for 2 days and then allowed to cool to room temperature. The precipitate was filtered off and washed with acetone. The filtrate was concentrated under vacuum. The product was purified by column chromatography on silica gel with DCM/petroleum ether 40-60 ºC (3/1 (v/v)) as an eluent. The product **12** was isolated as dark pink-red viscous liquid (0.20 g, 6 %).

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.04 (1H, br.s); 7.47 (1H, d, J=2.0); 7.20 (1H, dd, J_1=7.2; J_2=2.0); 6.89 (1H, d, J=7.2); 3.22 (2H, m); 2.89 (6H, s); 2.02 (1H, sept, J=6.8); 1.04 (6H, d, J=7.2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 141.45; 140.15; 131.81; 126.45; 114.96; 108.34; 50.92; 41.62; 28.43; 20.42. Anal. Calc. for $C_{12}H_{19}N_3O_2$ C, 60.74, H, 8.07; N, 17.71; found C, 60.59, H, 8.38, N, 17.94.

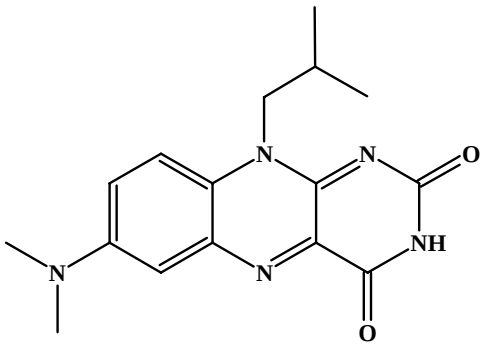
*N^1-Isobutyl-N^4,N^4-dimethylbenzene-1,2,4-triamine (**13**)*



Compound **12** (0.20 g, 0.8 mmol), palladium on carbon (5 %, 0.30 g) and ammonium formate (0.40 g) were stirred together in methanol (10 mL) for 30 min. Then additional portions of palladium on carbon (0.40 g) and ammonium formate (0.6 g) were added. After 10 min the reaction was complete. The solution was filtered from

palladium on carbon and the solvent was removed *in vacuo*. The obtained residue was dissolved in DCM and washed with water (2 x 100 mL) to remove excess of ammonium formate. The organic solvent was removed *in vacuo* to give a brown viscous liquid, which was used immediately in the next step.

*7-Dimethylamino flavin (**1**)*



Compound **13** (1 mmol), alloxan monohydrate (0.14 g, 1.0 mmol) and boron oxide (0.12 g, 1.5 mmol) were dissolved in glacial acetic acid (10 mL). The mixture was stirred at 80 ^{o}C for 3h. After cooling to room temperature, the solvent was removed *in vacuo* and the product was purified by column chromatography on silica gel using a solvent gradient (from pure DCM to acetone/DCM (15/85 v/v)) to afford **1** (0.02g, 8 % over two last steps).

M.p. 170 ºC (dec.). ^{1}H NMR (400 MHz, $CDCl_3$): δ. 8.86 (1H, br.s); 7.50 (1H, d, J=9.2); 7.37 (2H, m); 4.58 (2H, br. signal); 3.52 (6H, s); 2.42 (1H, m); 0.97 (6H, d, J=7.2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 209.23; 172.53; 168.33; 151.47; 124.93; 118.47; 110.17; 71.14; 52.71; 40.39; 30.72; 29.03; 20.26. IR (golden gate, ν, cm^{-1}): 3482.8; 3221.5; 2917.8; 2853.2; 1611.2; 1559.4; 1542.8; 1486.9; 1387.5; 1329.7; 1251.6; 1186.0; 808.0; 732.8; 639.3. Anal. Calc. for $C_{16}H_{19}N_5O_2$ C, 61.33, H, 6.11; N, 22.35; found C, 61.66, H, 6.28, N, 22.11.

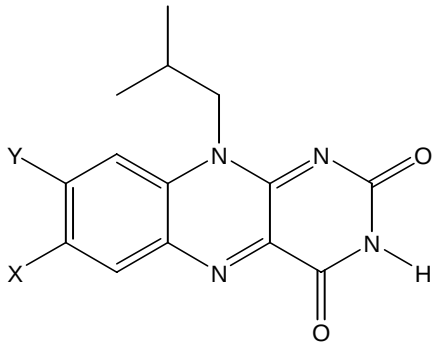
Compounds **2**[a], **3**[a], **4**[b] and **7**[c] were synthesised using previously described methods.

a. Y.-M. Legrand, M. Gray, G. Cooke and V. M. Rotello *J. Am. Chem. Soc*. 2003, **125**, 15789

b. G. Cooke, J. F. Garety, B. Jordan, N. Kryvokhyzha, A. Parkin, G. Rabani, V. M. Rotello. *Org. Lett.*, 2006, **8**, 2297.

c. E. Breinlinger, A. Niemz and V. M. Rotello, *J. Am. Chem. Soc.,* 1995, **117**, 5379.

LFER studies.



Equation 1: $\Delta G(X,Y) = \rho_m\sigma_m(X) + \rho_p\sigma_p(Y) + \Delta G(H,H)$,

where ρ_m = -6.9 kcal/mol/σ and ρ_p = -4.6 kcal/mol/σ

Fig S1. Showing how Equation 1 relates to structure of the flavins used in this study