Electronic Supplementary Information

Dendron-based model systems for flavoenzyme activity: towards a new class of synthetic flavoenzymes

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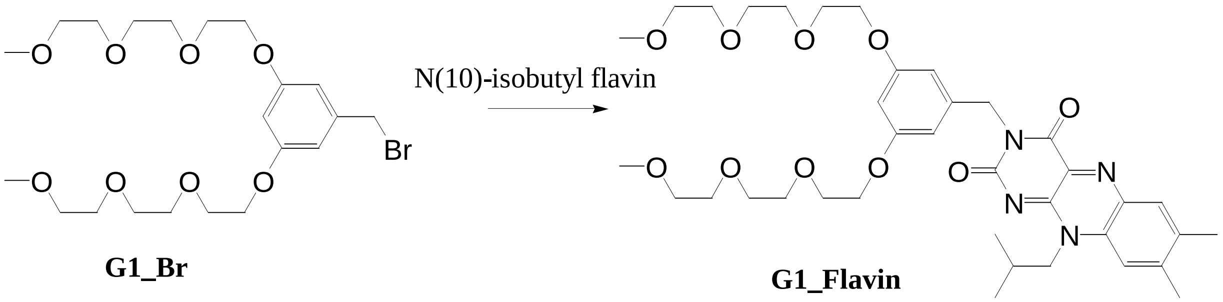
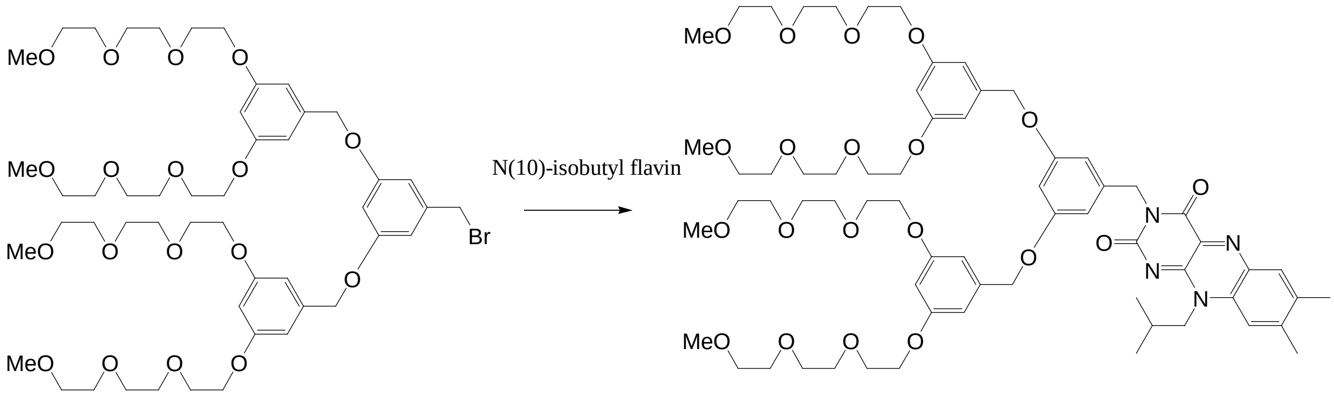
(a) Synthesis

G1-Flavin



To a solution of N(10)-isobutyl flavin (577 mg, 2.0 mmol) and potassium carbonate (1.16 g, 8.4 mmol) in acetonitrile (100 mL) was added **G1-Br** (1.98 g, 4.0 mmol). The mixture was stirred at room temperature for one week. The solid was then filtered off and the solvent removed under reduced pressure. The dark yellow/brown oil was then purified by flash chromatography (30 % acetone in DCM) to afford **G1_flavin** as an orange wax (1.04 g, 73 %): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.93 (6H, d, 2 x CH_3), 2.35 (3H, s), 2.38 (1H, m), 2.45 (3H, s), 3.28 (6H, s), 3.46 (4H, t), 3.52 (4H, t), 3.54 (4H, t), 3.62 (4H, t), 3.72 (4H, t), 3.99 (4H, t), 4.53 (2H, br d), 5.23 (2H, s), 6.29 (1H, s), 6.59 (2H, s), 7.32 (1H, s), 7.93 (1H, s) ppm; ^{13}C NMR (400 MHz, $CDCl_3$) δ 19.5, 20.0, 21.6, 27.4, 30.9, 45.1, 50.9, 53.5, 59.0, 67.4, 69.6, 70.5, 70.6, 70.7, 71.9, 101.2, 108.1, 115.8, 131.5, 132.7, 134.9, 135.9, 136.5, 139.0, 147.5, 149.2, 155.4, 159.7, 159.9; EI (*m/z*) calcd for $C_{37}H_{52}N_4O_{10}$ 713.38 found 713.38.

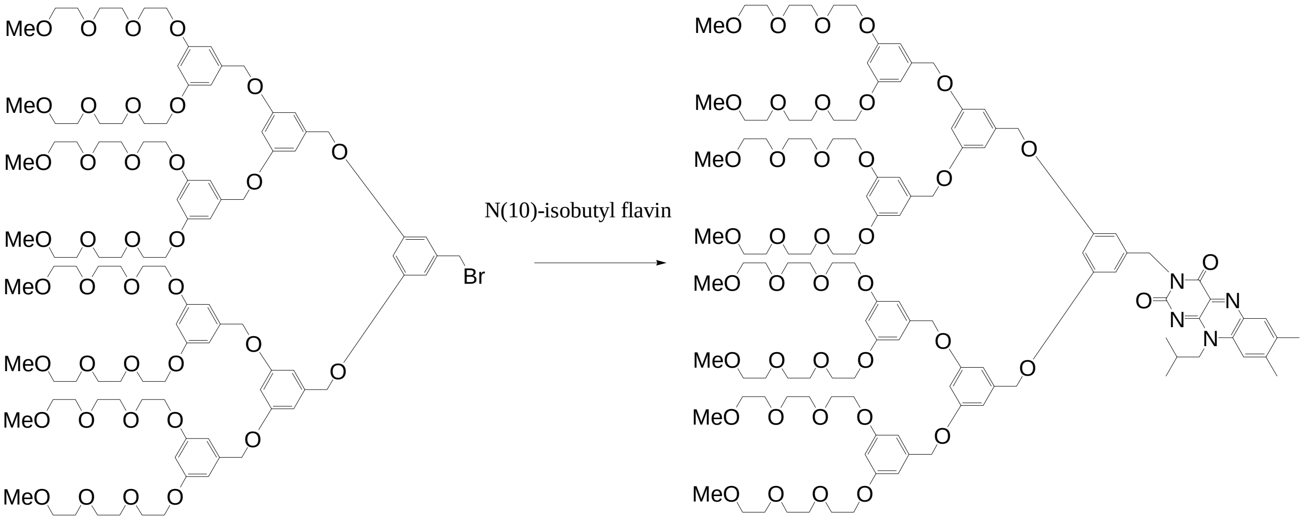
G2-Flavin



G2_Br **G2_Flavin**

To a solution of N(10)-isobutyl flavin (120 mg, 0.4 mmol) and potassium carbonate (415 mg, 3.0 mmol) in acetonitrile (20 mL) was added **G2_Br** (0.4 g, 0.4 mmol). The mixture was stirred at room temperature for eight days. The solid was then filtered off, and washed with THF (50 mL). The washings and the filtrate were combined and the solvent removed under reduced pressure. The dark yellow/brown oil was then purified by flash chromatography (25 % acetone in DCM initially and then gradually increased to 75% acetone in DCM) to afford **G2_flavin** as an orange oil (140 mg, 28 %): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.93 (6H, d, 2 x CH_3), 2.36 (3H, s, CH_3), 2.40 (1H, m, $\underline{CH}(CH_3)_2$), 2.56 (3H, s, CH_3), 3.30 (12H, s, OCH_3), 3.46 (8H, m, OCH_2), 3.57-3.61 (16H, m, $O(CH_2)_2O$), 3.65 (8H, m, OCH_2), 3.76 (8H, m, OCH_2), 4.02 (8H, m, OCH_2), 4.34 (2H, br s, *N*-CH_2), 4.85 (4H, s, CH_2), 4.87 (2H, s, CH_2), 6.51 (4H, d, *J* 2.2, ar CH), 6.59 (2H, d, *J* 2.2, ar CH), 6.69 (1H, d, *J* 2.2, ar CH), 6.80 (2H, d, *J* 2.2, ar CH), 7.32 (1H, s, CH), 7.93 (1H, s, CH) ppm; ^{13}C NMR (400 MHz, $CDCl_3$) δ 19.5, 20.1, 21.7, 27.5, 45.3, 50.7, 59.1, 67.5, 69.7, 69.9, 70.6, 70.7, 70.8, 71.9, 101.3, 101.6, 106.1, 108.4, 115.8, 132.8 ppm. Found: C, 62.44; H, 7.46; N, 4.51. $C_{65}H_{92}N_4O_{20}$ requires: C, 62.48; H, 7.42; N, 4.48%. FAB-MS: m/z =1250.

G3-Flavin



G3_Br

G3_Flavin

A solution of **G3_Br** (172 mg, 0.08 mmol), N(10)-isobutyl flavin (52 mg, 0.17 mmol), potassium carbonate (55 mg, 0.4mmol) and 18-crown-6 (10 mg, 0.04 mmol) in acetonitrile (10 ml) was stirred at room temperature for seven days. The solid was then filtered and washed with THF (20 mL). The washings and filtrate were then combined and the solvent removed under reduced pressure. The crude residue was then purified *via* flash chromatography (25% acetone/DCM increased to acetone/DCM) to afford **G3_flavin** as a yellow oil (23 mg, 12%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.94 (6H, d, 2 x CH_3), 2.34 (3H, s, CH_3), 2.38 (1H, m, CH), 2.49 (3H, s, CH_3), 3.35 (24H, s, OCH_3), 3.48 (16H, m), 3.55 – 3.62 (32H, m), 3.65 (16H, t), 3.74 (16H, t) 4.03 (16H, t), 4.51 (2H, s, CH_2-N), 4.88 (8H, s, CH_2), 4.90 (4H, s, CH_2), 5.17 (2H, s, CH_2), 6.37 (4H, t, CH), 6.43 (2H, d, CH) 6.47 (1H, t, CH), 6.52 (8H, t, CH), 6.59 (4H, d, CH) 6.82 (2H, d, CH), 7.31 (1H, s, CH), 7.94 (1H, s, CH) ppm; ^{13}C NMR (400 MHz, $CDCl_3$) δ 21.0, 31.0, 59.1, 67.5, 69.7, 70.6, 70.7, 70.8m, 71.9, 76.7, 77.1, 77.4, 102.0, 132.3, 141.0, 106.1 ppm. Found: C, 62.56; H, 7.50; N, 2.43. $C_{121}H_{172}N_4O_{40}$ requires: C, 62.57; H, 7.46; N, 2.41%.

N(10)-Isobutyl flavin was prepared according to: A. Niemz, J. Imbriglio and V. M. Rotello, *J. Am. Chem. Soc.*, 1997, **119**, 887.
G1_Br to **G3_Br** were prepared according to: M. J. Hannon, P. C. Mayers and P . C. Taylor *J. Chem. Soc., Perkin Trans 1*, 2000, 1881.

(b) Molecular modeling.

Molecular dynamic simulations were performed on the flavin dendrimers using MacroModel (Maestro v8.0) from Schrodinger, Inc. Amber* force field was chosen from the subsystem "Dynamics" and the calculations were performed in a continuum of water as the solvent. Electrostatic treatment was set to the constant dielectric setting of 1.0. The external cutoffs for the continuum of water were automatically set to 8.0 (Van der Waals), 20.0 (electrostatics), and 4.0 (hydrogen bonding). There were no constraints placed on the molecule. The method used was TNCG (Truncated Newton linear Conjugate Gradient), with the maximum number of iterations at 500. Convergence was based on a gradient and the threshold was set to 0.0500. Stochastic dynamics were used, and all the bonds were shaken during the calculation. The simulation temperature was performed over the range of 1000K-100K (decreasing). The time steps (fs) were set to 1.5, the equilibrium time (ps) set to 1.0, and the simulation time (ps) set to 10.0.

(c) Fluorescene measurements

Solutions of flavins (5 μmol dm^{-3} , calibrated using UV) in buffer:isopropanol (95:5) were used and the fluorescence spectra were recorded using a SpectraMax M5, Molecular Devices.

(d) Electrochemistry

Cyclic voltammetry experiments were performed using a CH Instruments 440A electrochemical workstation. A three electrode configuration was used with a platinum disc working electrode, a platinum wire counter electrode and a silver/ silver chloride reference electrode. The solution was purged with nitrogen prior to recording the electrochemical data, and all measurements were recorded under a nitrogen atmosphere.

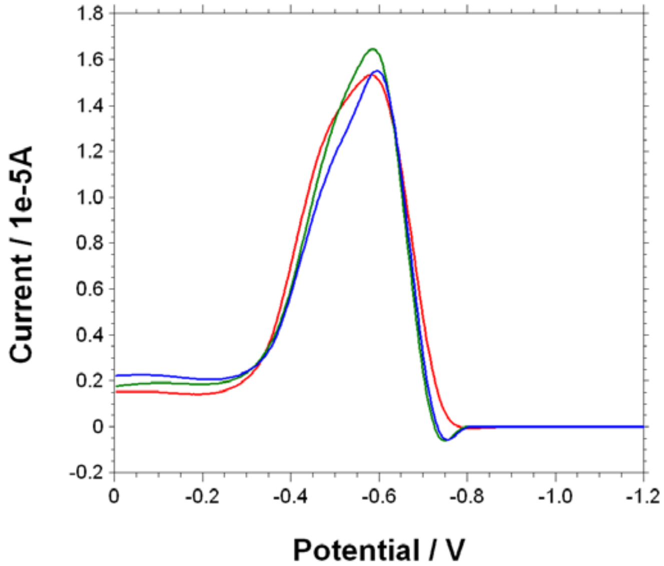


Fig. S1: Square wave voltammetry of **G1_flavin** (RED LINE), **G2_flavin** (BLUE LINE) and **G3_flavin** (GREEN LINE) in pH = 8.0 phosphate buffer/isopropanol 95:5 (4x10^{-5} M). E = -0.6 V versus Ag/AgCl.

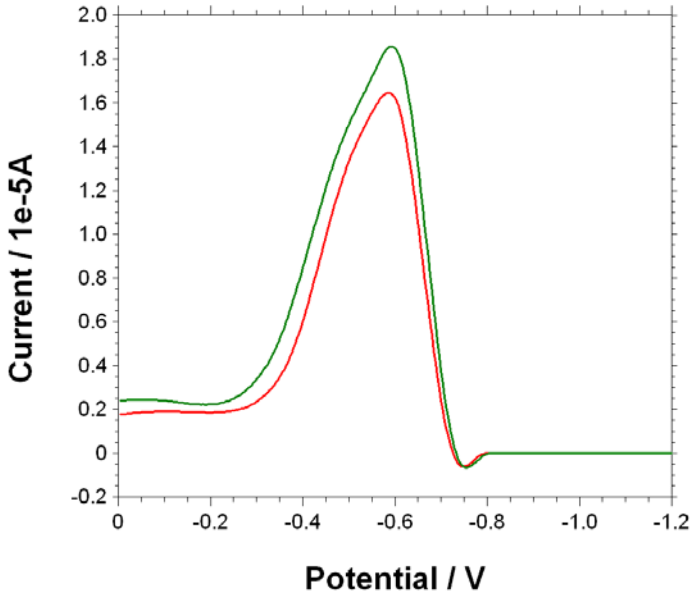


Fig. S2: Square wave voltammetry of **G3_flavin** (RED LINE) and **riboflavi**n (GREEN LINE) in pH = 8.0 phosphate buffer/isopropanol 95:5 (4x10^{-5} M). E = -0.6 V versus Ag/AgCl.

(e) Catalytic activity of the flavin-containing dendrons

Catalytic activity assays were performed in sodium phosphate pH 8 buffer/ethanol (95:5) (5 mM,) at 30 °C with flavin concentration of 5 μmol dm^{-3} and varied substrate (**BNAH**) concentrations. The **BNAH** concentrations were varied from 0.1 - 0.8 mmol with an increment of 0.1 mmol (*i.e.* 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, and 0.8 mmol).

Oxidation activity of flavin was followed by monitoring the decrease in absorbance of **BNAH** at at 358 nm (intervals of 15 s for 15 min) with a microplate reader (SpectraMax M5, Molecular Devices). The assays were performed in triplicate, and the averages were used for the kinetic analysis. The decrease in absorbance at 358 nm corresponding to the disappearance of **BNAH** from the reaction medium was converted to a concentration scale using a molar extinction coefficient of 4675.25 mole dm^{-3} cm^{-1} for **BNAH**. The concentration scale was used to determine the initial velocities within the linear range of 15 min.