Electronic Supplementary Information (ESI)

Model systems for flavoenzyme activity: a tuneable intramolecularly hydrogen bonded complex

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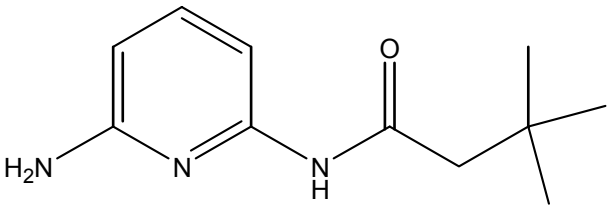
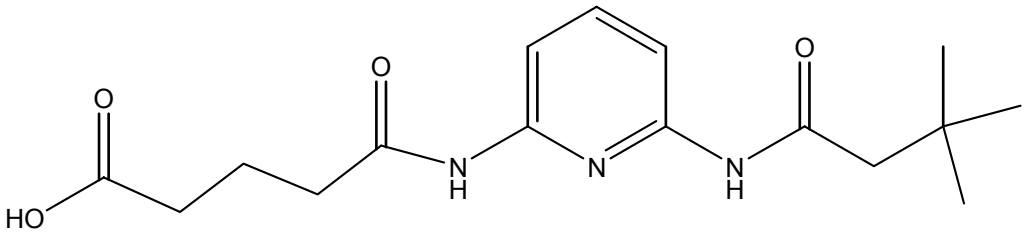
Synthesis of *N*-(6-amino-pyridin-2-yl)-2,3-dimethyl-butyramide



2,6-Diaminopyridine (15.41 g, 141.4 mmol) was dissolved in a mixture of CH_2Cl_2 (600 mL) and NEt_3 (20 mL). After purging the solution with nitrogen, *tert*-butylacetyl chloride (20 mL, 141.4 mmol) was added dropwise over a period of thirty minutes. The solution was then allowed to stir at room temperature for 18 h under N_2. The solvent was removed under reduced pressure and THF (200 mL) was added to the brown residue. Filtration of this suspension removed the triethylamine hydrochloride salt. The solution was then concentrated to give a brown solid, which was purified using column chromatography (silica gel, eluting with ethyl acetate) to afford **1** as a white solid (20.28 g, 71 %). Mp 96-98 °C; ν_{max} (KBr): 3470.9, 3442.21, 3367.6, 3248.5, 3059.6, 2952.1, 2906.4, 2865.3, 1950.9, 1654.4, 1610.1, 1542.0, 1462.4, 1365.2, 1355.5, 1327.5, 1300.6, 1287.1, 1235.7, 1201.9, 1162.8, 1138.9, 1076.0, 1042.2, 986.0, 947.2, 909.5; ^{1}H NMR (200 MHz, $CDCl_3$) δ = 7.75 (1H, s), 7.55 (1H, d), 7.50 (1H, t), 7.63 (1H, d), 4.40 (2H, s), 2.30 (2H, s), 1.15 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$) 170.5, 157.5, 150.0, 140.0, 104.5, 103.5, 52.0, 51.5, 30.0; MS (EI) m/z = 207 (M^+). Elemental analysis cald. (%) for $C_{11}H_{17}N_3O$: C 63.74, H 8.27, N 20.27; found: C 63.66, H 8.19, N 20.17.

Synthesis of 4-[6-(3,3-dimethyl-butyrylamino)-pyridin-2-ylcarbamoyl]-butyric acid



N-(6-Amino-pyridin-2-yl)-2,3-dimethyl-butyramide (1.06 g, 5.1 mmol) and glutaric anhydride (1.18 g, 10.4 mmol) were dissolved in pyridine (75 mL) . The solution was then heated under reflux for 20 h. The solution was concentrated under reduced pressure to yield a dark brown oil. The subsequent addition of CH_2Cl_2 (100 mL) resulted in the precipitation of a white solid, which was removed by filtration and washed

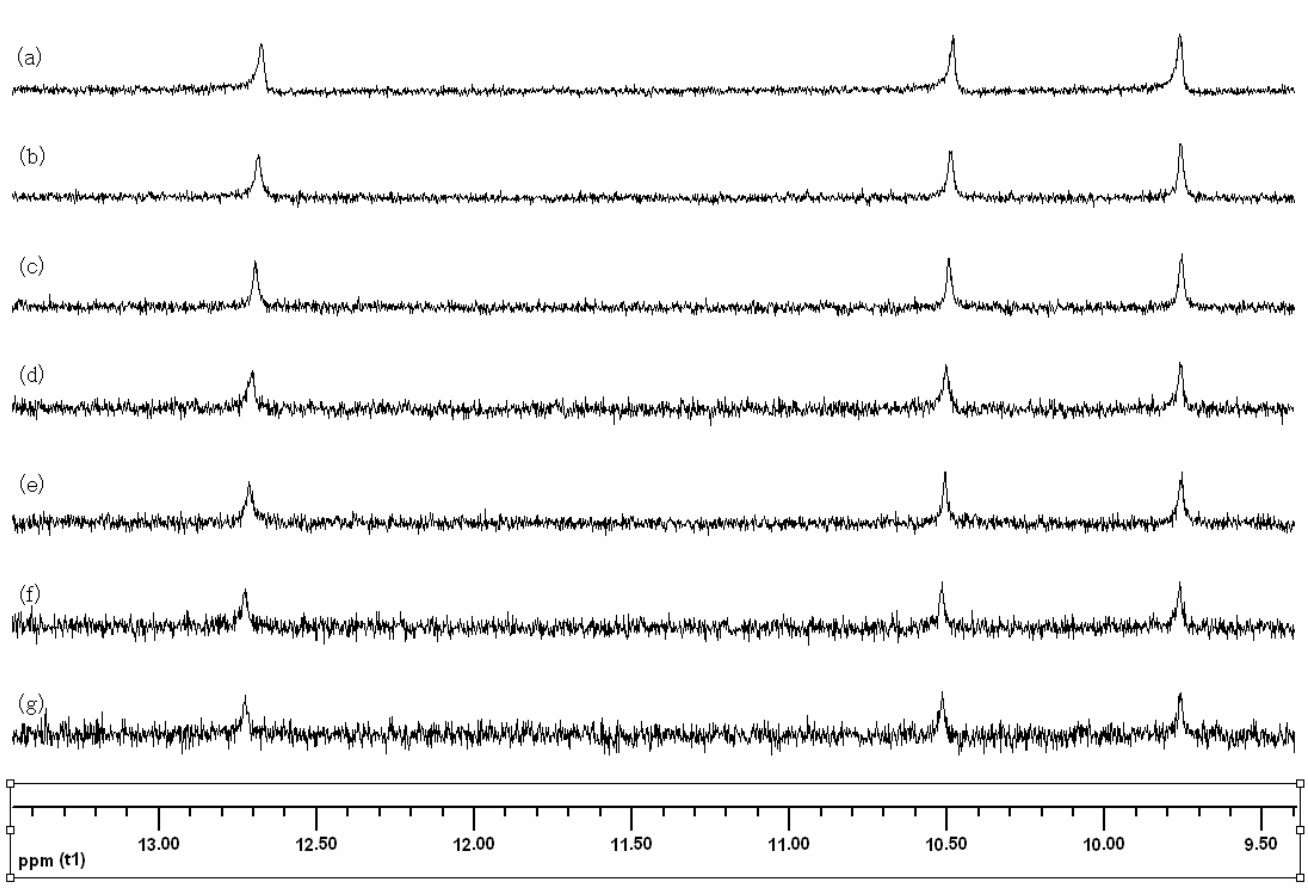
with CH_2Cl_2 (50 mL) and dried under high-vacuum. The product was obtained as a white powder (1.14 g, 62%). Mp 167-169 °C; ν_{max} (KBr): 3284.5, 3203.0, 3058.1, 2967.5, 1704.9, 1668.7, 1614.3, 1587.2, 1541.9, 1455.8, 1369.8, 1329.1, 1288.3, 1215.8, 1161.5; ^{1}H NMR (200 MHz, CD_3OD) δ = 7.55 (3H, m), 2.45 (4H, m), 2.25 (2H, s), 1.95 (2H, m), 1.05 (9H, s); MS (EI) m/z = 321 (M^+).

Synthesis of **3**

4-[6-(3,3-Dimethyl-butyrylamino)-pyridin-2-ylcarbamoyl]-butyric acid (0.87 g, 2.7 mmol), *N*-(10)-hydroxyhexyl flavin* (0.1845g, 0.54 mmol), EDCI (0.56 g, 2.70 mmol) and DMAP (1.01 g, mmol) were dissolved in dry DMF (25 mL), and the resulting solution was stirred under N_2 at room temperature for three days. The reaction mixture was then filtered, and the filtrate was concentrated under reduced pressure. The resulting residue was purified using column chromatography (silica gel, eluting with CH_2Cl_2/acetone, 9:1 v/v) to afford **3** as a yellow solid (0.15 g, 43%). Mp 198 °C $_{dec}$; ν_{max} (KBr): 3421.0, 3276.3, 3208.9, 3054.5, 2954.2, 2867.6, 1698.6, 1670.3, 1583.7, 1546.7, 1448.2, 1401.8, 1352.1, 1276.1, 1242.5, 1199.5, 1176.0, 1155.8, 1135.0, 1012.7, 808.5. ^{1}H NMR (200 MHz, $CDCl_3$) δ = 12.50 (1H, s), 10.35 (1H, s), 9.75 (1H, s), 8.00 (1H, s), 7.95 (2H, d), 7.65 (1H, d), 7.40 (1H, d), 4.70 (2H, m), 2.75 (2H, t), 1.05 (9H, s); MS (ES) m/z = 646 [M+H]. Elemental analysis cald. (%) for $C_{34}H_{43}N_7O_6$: C 63.24, H 6.71, N 15.18; found: C 63.64, H 6.92, N 15.17.

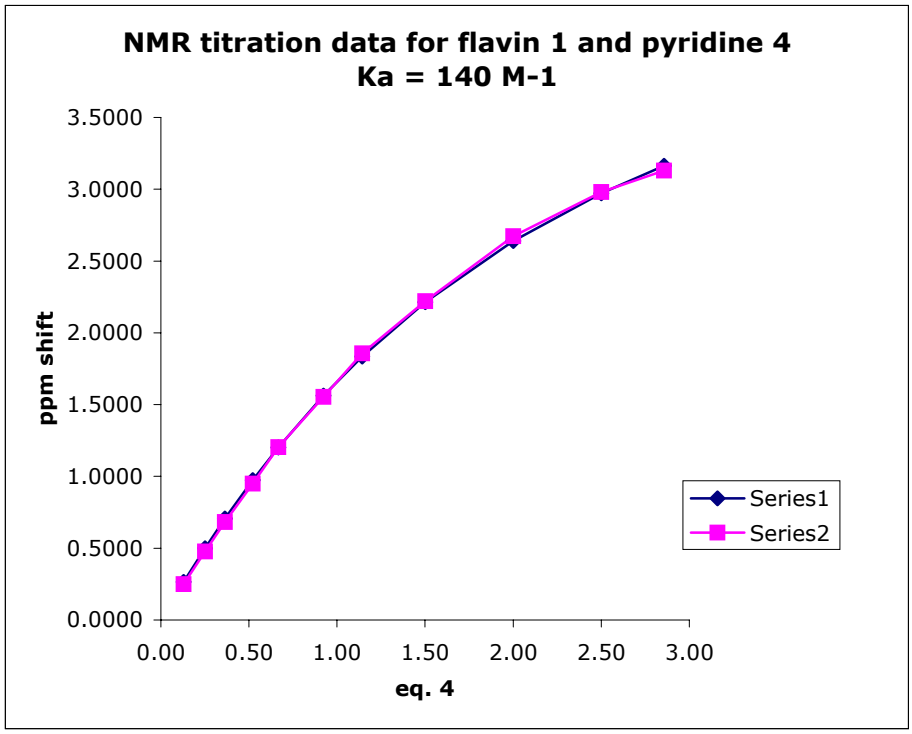
* C. Frier, J.-L. Décout and M. Fontecave, *J. Org. Chem.*, 1997, **62**, 3520.

^{1}H NMR spectra of **3** recorded at different concentrations in $CDCl_3$ at 25°C

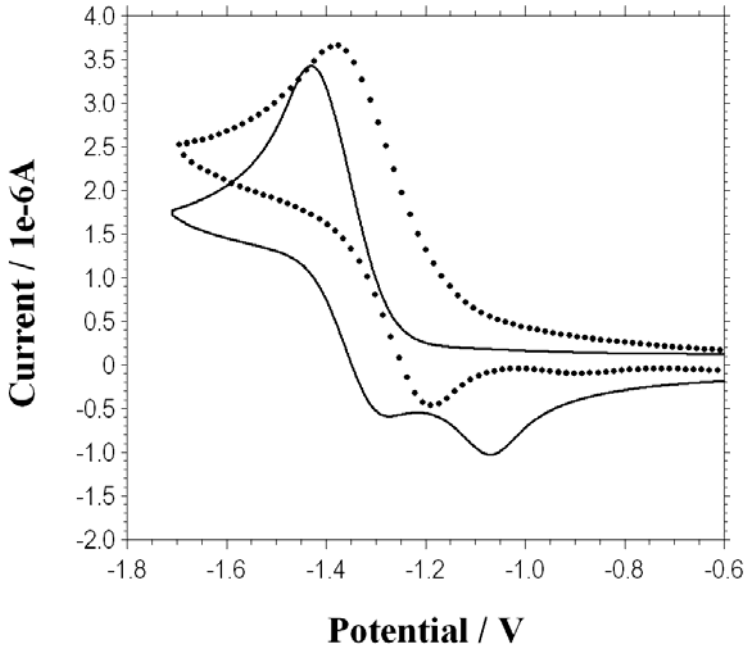


Selected 200 MHz ^{1}H NMR spectra of **3** recorded in $CDCl_3$ recorded at the following concentrations: (a) 1.2×10^{-2} M; (b) 7.7×10^{-3} M; (c) 5.8×10^{-3} M; (d) 3.8×10^{-3}; (e) 2.9×10^{-3}; (f) 1.8×10^{-3}; (g) 1.5×10^{-3} M.

NMR titration data for **1.4** recorded in $CDCl_3$



CV data for compound **1** upon the addition of excess **4**.



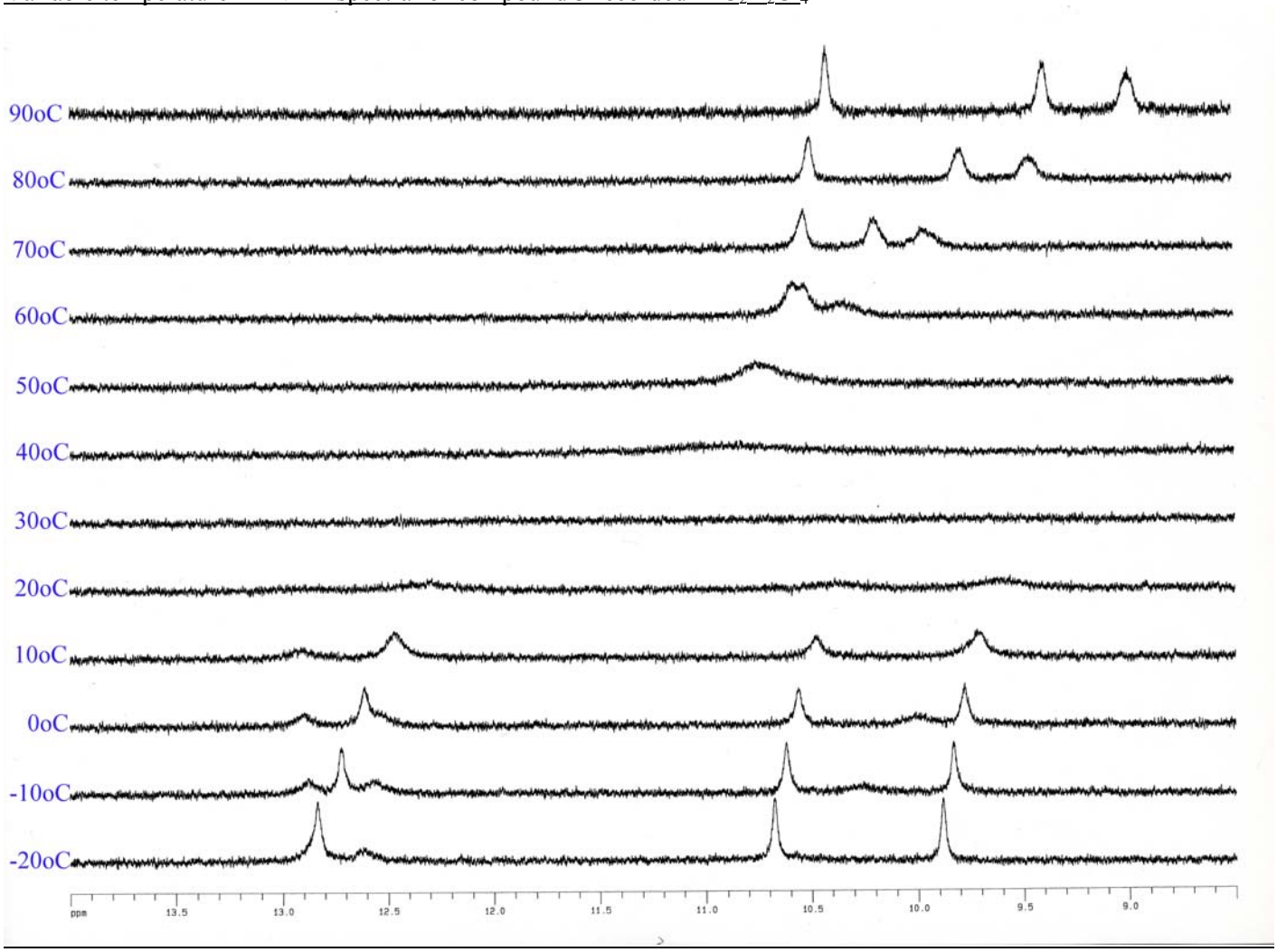
CV of **1** in CH_2Cl_2 (~ 4×10^{-4} M) (—) and upon the addition of excess **4** (~ 2×10^{-2} M) (·····).

$E_{½}$ of **1** before addition of **4** = -1.36 V.

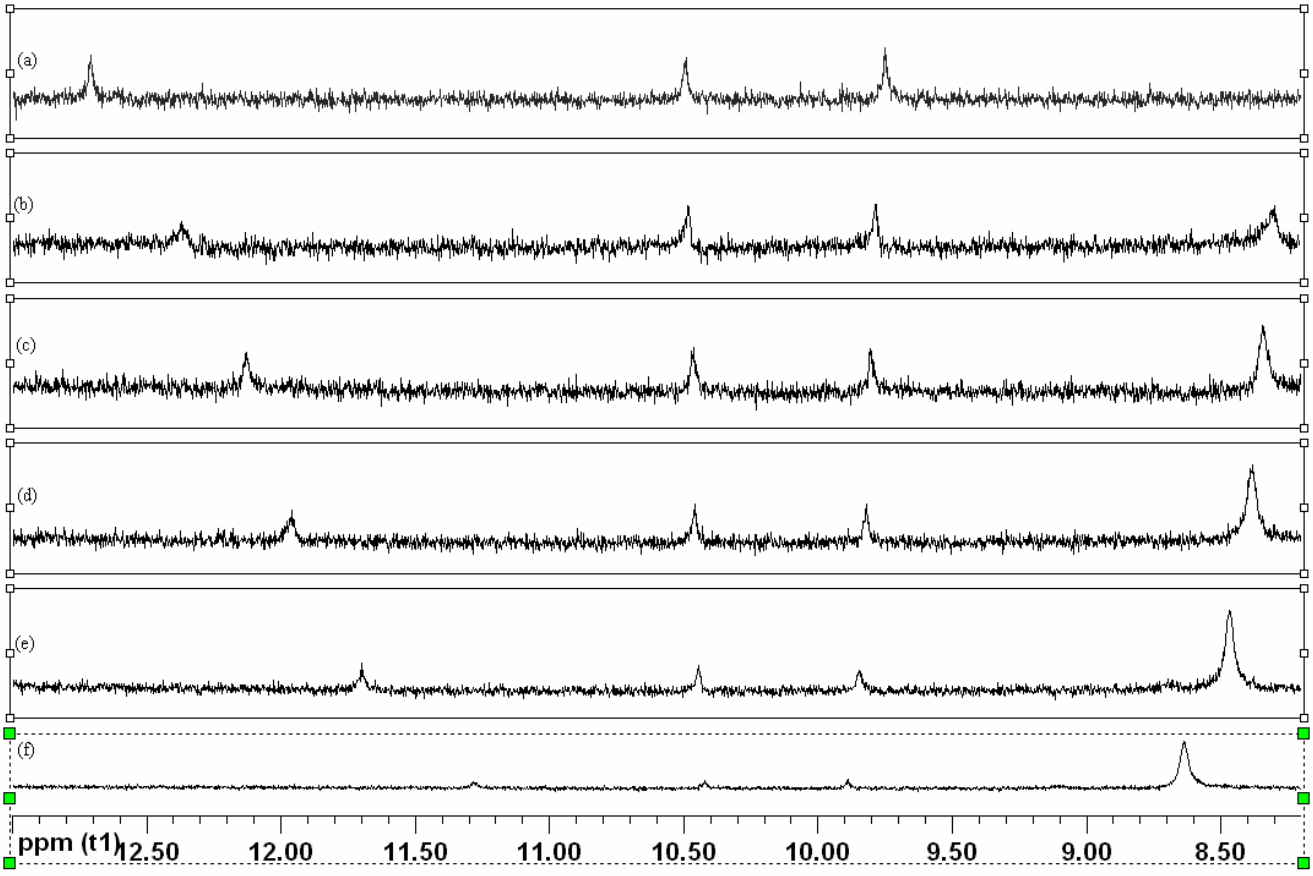
$E_{½}$ of **1** after the addition of **4** = -1.28 V.

Peak at -1.07 disappears.

Variable temperature ^{1}H NMR spectra for compound **3** recorded in $C_2D_2Cl_4$



^{1}H NMR spectra of **3** recorded in $CDCl_3$ at 25°C in the presence of various equivalents of **5**



Selected 200 MHz ^{1}H NMR spectra of **3** (7×10^{-4} M in $CDCl_3$) recorded in the presence of the following equivalents of **5**: (a) 0; (b) 1; (c) 3; (d) 7; (e) 12; (f) 24.