Supporting Information

'Lock and Key' Control of Optical Properties in a Push-Pull System

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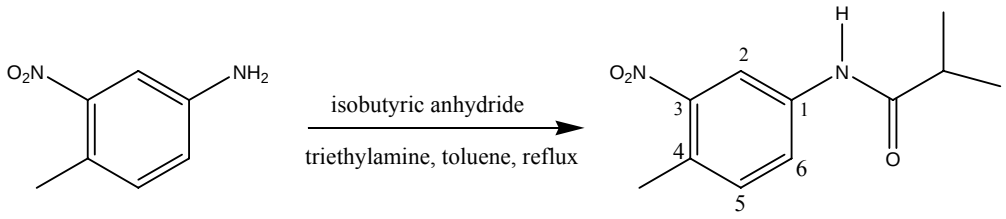
Materials and General Methods

Reagent grade tetrahydrofuran was dried via distillation over sodium. All other reagents and solvents utilized in reactions described thereinafter are of laboratory reagent grade and were used as received without further purification. NMR Spectra were recorded on a Bruker AC 200 instrument at 200.1 MHz for ^{1}H and 50.3 MHz for ^{13}C NMR, respectively on a Bruker DPX 400 instrument at 100.6 MHz for ^{13}C NMR (unless otherwise specified). Melting points were measured on a Stuart Scientific SMP10. Low resolution molecular masses were determined on a Kratos Concept 1S which was operated in Electron Impact EI mode. Electrospray ESI experiments were performed using a Waters ZQ4000 and Electron Impact EI experiments were recorded on a Micromass Quattro II instrument. FTIR – Spectra were measured on an Elmer Perkin Spectrometer. The samples were prepared as KBr discs.

Synthesis

N-(4-methyl-3-nitrophenyl)isobutyramide 2



1 **2**

A stirring solution of 4-methyl-3-nitroaniline **1** (10 g, 65.7 mmol), triethylamine (66.6 ml, 0.48 mol, 7.3 eq.) and isobutyric anhydride (47.3 ml, 0.29 mol, 4.4 eq.) in toluene (112 ml) was heated to reflux under an atmosphere of nitrogen. The reaction progress was monitored by means of TLC (ethyl acetate/petrol 1:4). After 48 hours heating was stopped. The solvent was evaporated under reduced pressure to give a dark-brown liquid residue. The crude product was dissolved in dichloromethane (150 ml), transferred to a separation funnel and washed twice with saturated aqueous sodium bicarbonate solution (2 x 100 ml) and once with water (100 ml). The organic layer was dried over anhydrous magnesium sulphate. The solvent was removed under reduced pressure to give a concentrated yellow liquid residue. This was stored in the fridge. Dirty yellow crystals formed overnight which were recrystallized from boiling methanol (approx. 8 ml) [a mixture with a nonsolvent like hexane might be much better]. The recrystallized product was separated from the solution by filtration, transferred into a conical flask and suspended with hexane to remove last traces of other products. Finally the pale yellow crystals were collected by filtration. The desired *N*-(4-methyl-3-nitrophenyl)isobutyramide **2** (11.17 g, 50.3 mmol, 77 %) was obtained in a good yield.

Melting Point 112 – 113°C.

^{1}H NMR analysis: δ_H(200.13 MHz, $CDCl_3$)/ppm 8.14 (d, 1 H, C2H), 7.77 (dd, 1 H, C6H), 7.46 (bs, 1 H, NH), 7.27 (d, 1 H, C5H), 2.54 (sept., 1 H, CH_3*CH*CH_3), 2.54 (s, 3 H, C_{ar}-*CH*$_3$), 1.26 (d, 6 H, *CH*$_3$CH*CH*$_3$).

^{13}C NMR analysis: δ_C(50.3 MHz, $CDCl_3$)/ppm 175.6 (C=O), 148.9 (C_{ar}), 136.9 (C_{ar}), 133.1 (C_{ar}), 128.9 (C_{ar}), 124.3 (C_{ar}), 115.6 (C_{ar}), 36.6 (CO-*CH*-$(CH_3)_2$), 20.0 (C_{ar}-*CH*$_3$), 19.5 (*CH*$_3$CH*CH*$_3$).

ν_{max}(KBr)/cm^{-1} 3285*s* (NH), 3105*w* (CH_{ar}), 2967*m* (CH_{alk}), 2933*m* (CH_{alk}), 2874*m* (CH_{alk}), 1661*s* (NH-*C*=*O*), 1590*m* (aromatic C=C), 1535*s* (C-NO_2), 1449*m*, 1386*w*, 1336*s* (C-NO_2),

1304*m*, 1244*m*, 1200*m*, 1162*m*, 1100*m*, 1066*w*, 958*m*, 894*m*, 842*m* and 820*m* (1,3,4-trisubstituated aromatic ring), 757*m*, 690*m*.

m/z (EI/MS) 222 (47%, M^+), 179 (16%, M^+-C_3H_7), 152 (58%, M^+-C_4H_6O), 135 (55%, M^+-C_4H_9NO), 104 (48%), 77 (46%).

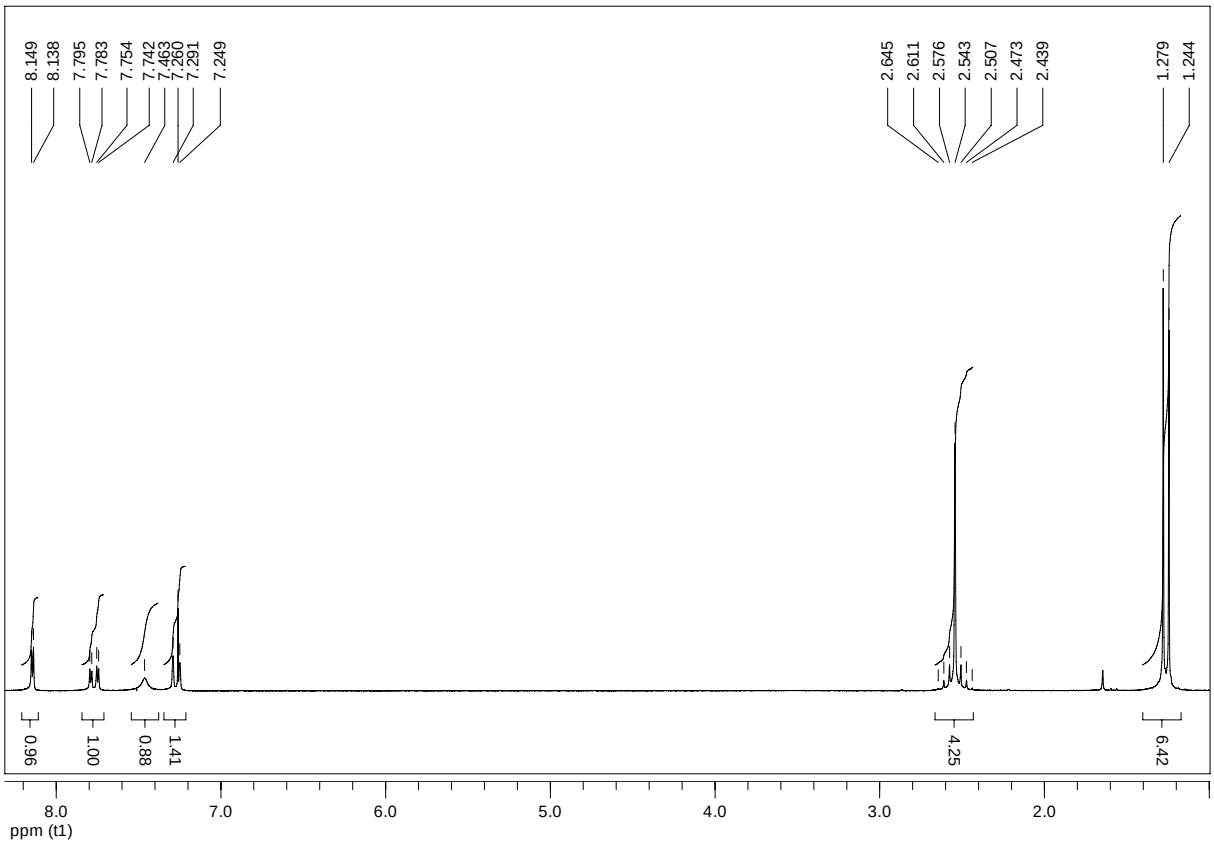


Fig. S1 ^{1}H NMR spectra of ***N*-(**4-methyl-3-nitrophenyl)isobutyramide **2**

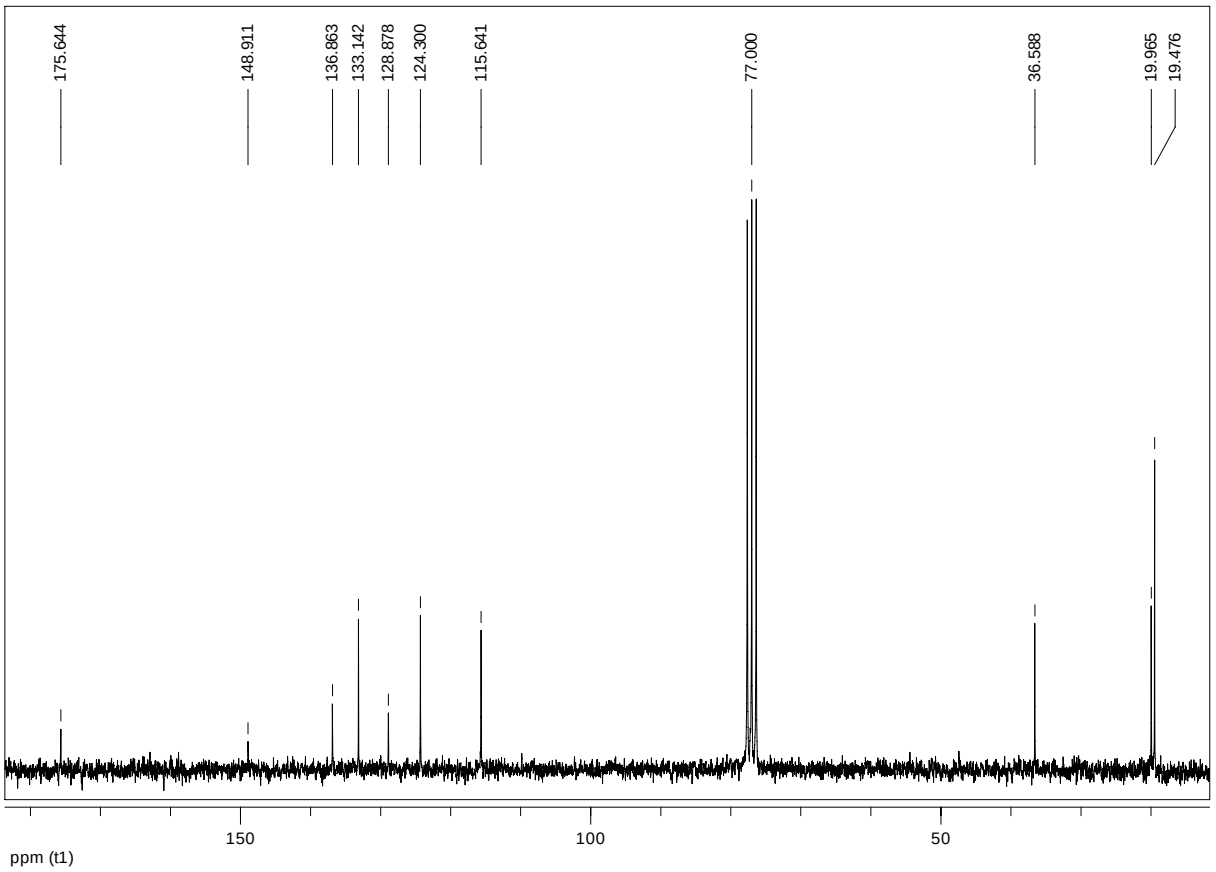
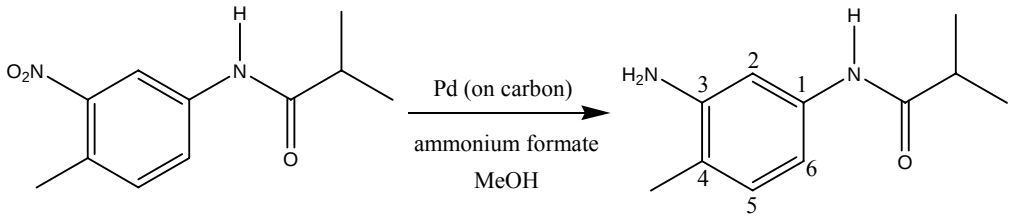


Fig. S2 ^{13}C NMR spectra of ***N*-(**4-methyl-3-nitrophenyl)isobutyramide **2**

***N*-(3-amino-4-methylphenyl)isobutyramide 3**



2 **3**

To a stirring solution of ammonium formate (9.06 g, 0.138 mol, 10 eq.) in methanol (190 ml) a catalytic amount of Pd on carbon (100.4 mg) was added. The reaction flask was then flushed once with nitrogen before the starting material *N*-(4-methyl-3-nitrophenyl)isobutyramide **2** (3.06 g, 13.77 mmol, 1 eq.) was introduced. The mixture was stirred for 90 minutes at room temperature before the solution was filtered to remove the Pd - catalyst. The solvent was evaporated under reduced pressure to give a white solid residue. This residue was then suspended in DCM (190 ml) and heated up to 40°C for 30 minutes to separate the soluble product from the insoluble ammonium formate. The solution was separated from the solid by

filtration, dried over magnesium sulphate. Finally the solvent was removed under reduced pressure to give the pure product *N*-(3-amino-4-methylphenyl)isobutyramide **3** (2.64 g, 13.75 mmol, 100 %).

Melting Point: 160 – 161 °C

^{1}H NMR analysis: δ_H(200.13 MHz, $CDCl_3$)/ppm 7.20 (d, 1 H, C2H), 7.17 (bs, 1 H, NH), 6.94 (d, 1 H, C5H), 6.61 (dd, 1 H, C6H), 3.60 (bs, 2 H, NH_2), 2.46 (sept., 1 H, CH_3*CH*CH_3), 2.11 (s, 3 H, C_{ar}-*CH*$_3$), 1.22 (d, 6 H, *CH*$_3$CH*CH*$_3$).

^{13}C NMR analysis: δ_C(50.3 MHz, $CDCl_3$)/ppm 175.1 (C=O), 145.0 (C_{ar}), 137.0 (C_{ar}), 130.5 (C_{ar}), 118.1 (C_{ar}), 109.6 (C_{ar}), 106.5 (C_{ar}), 36.7 (CO-*CH*-$(CH_3)_2$), 19.6 (C_{ar}-*CH*$_3$), 16.8 (*CH*$_3$CH*CH*$_3$).

ν_{max}(KBr)/cm^{-1} 3455*s* (NH_2), 3366*s* (NH_2), 3289*s* (NH), 3130*w* (CH_{ar}), 2970*m* (CH_{alk}), 2930*w* (CH_{alk}), 2871*w* (CH_{alk}), 1682*s*, 1658*s* (Amide CO), 1626*m*, 1600*s*, 1547*s* (Amide CO), 1455*m*, 1426*s*, 1238*s*, 1101*m*, 942*m*, 874*m*, 803*s*, 709*s*.

m/z (EI/MS) 192 (89%, M^+), 149 (6%, M^+-C_3H_7), 122 (100%, M^+-C_4H_6O).

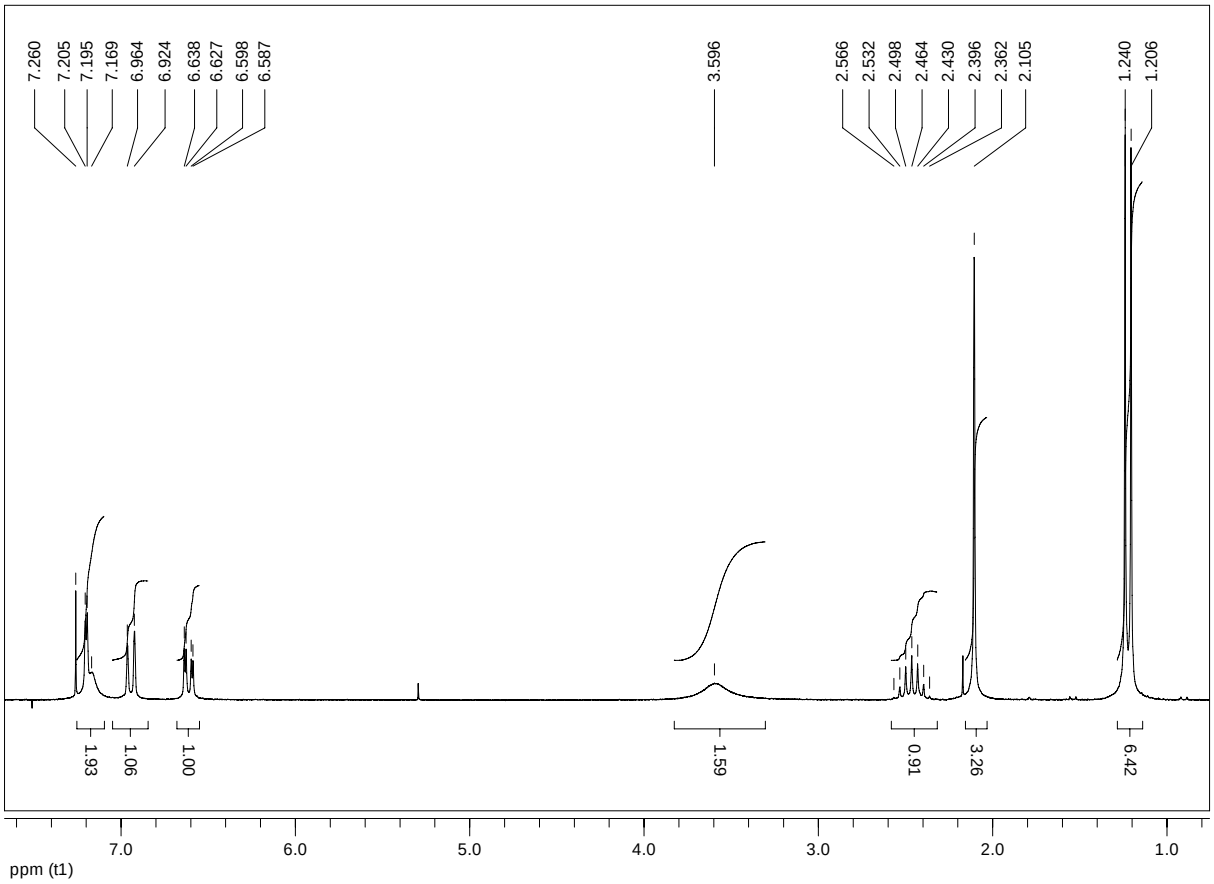


Fig. S3 ^{1}H NMR spectra of *N*-(3-amino-4-methylphenyl)isobutyramide **3**

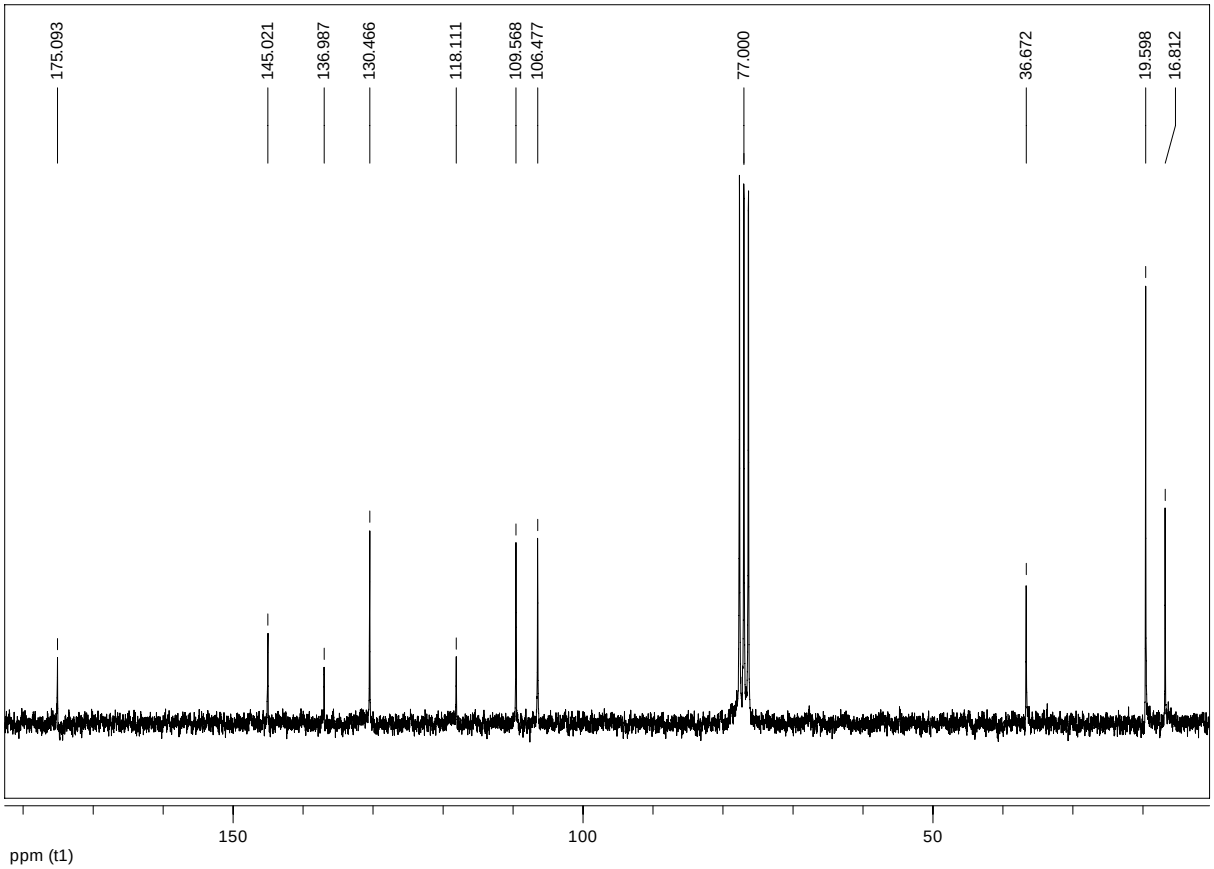
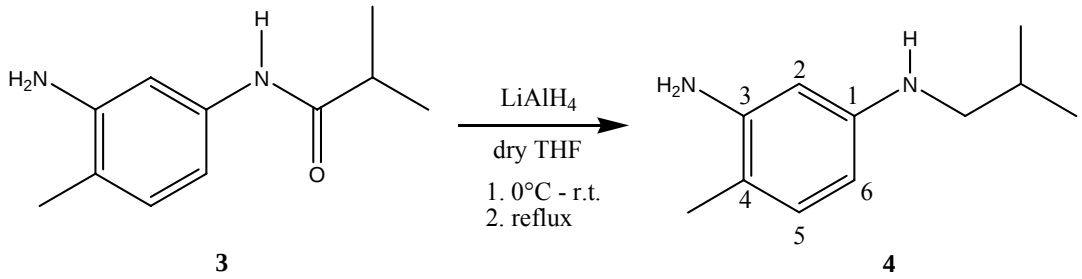


Fig. S4 ^{13}C NMR spectra of *N*-(3-amino-4-methylphenyl)isobutyramide **3**

N[1]-isobutyl-4-methylbenzene-1,3-diamine 4

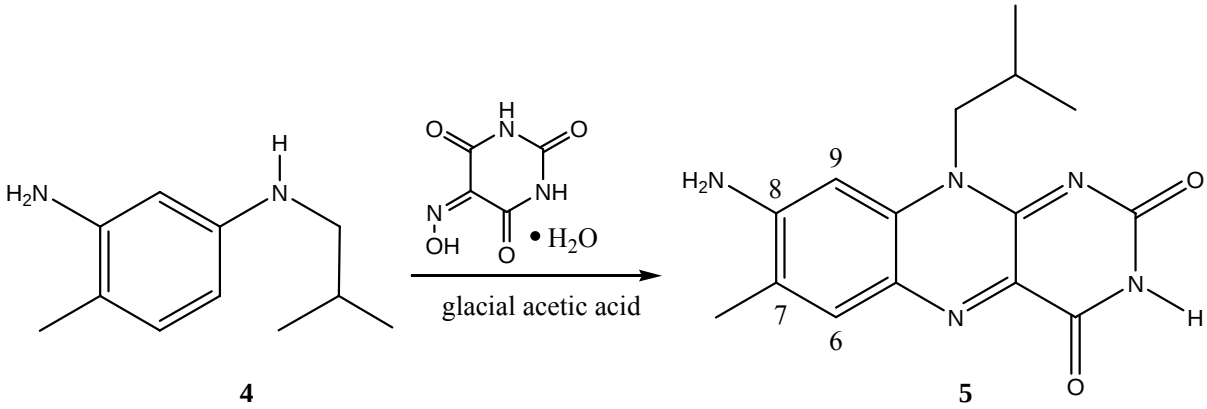


3 **4**

A dry 500 ml round-bottomed-flask was charged with dry THF (200 ml) under nitrogen atmosphere. The solvent was then cooled down to 0°C in an ice-bath, before $LiAlH_4$ (3.01 g, 79.3 mmol, 4.3 eq.) was added. To this stirring suspension the starting material *N*-(3-amino-4-methylphenyl)isobutyramide **3** (3.51 g, 18.27 mmol, 1 eq.) was added. The ice-bath was removed after complete addition of the starting material. The stirring reaction mixture was left at room temperature over night. TLC analysis (petrolether : ethyl acetate 1 : 1) the next morning showed only little conversion. Suspecting the $LiAlH_4$ was too old and therefore unreactive, another portion of new $LiALH_4$ (2.77 g, 73.06 mmol, 4 eq.) was added once again to the cooled down stirring reaction mixture. Afterwards the reaction was heated to reflux for 4 hours. TLC analysis showed now an approximately conversion of 80 %. After stirring the reaction mixture at room temperature over night again the remaining $LiAlH_4$ was deactivated by dropwise addition of water (7 ml), aqueous sodium hydroxid (15 wt%, 7 ml) and another amount of water (14 ml). Due to the big excess of $LiAlH_4$ the mixture became very viscous and difficult to stir, thus some THF was added.

The white precipitate that formed during the aqueous workup was separated from the solution by suction filtration and washed intensively with diethylether (150 ml). The solution was afterwards dried over magnesium sulphate, then the solvent was evaporated under reduced pressure to give the dark brown liquid crude product *N*[1]-isobutyl-4-methylbenzene-1,3-diamine **4** (3.13 g). No further purification steps and no analyses were done, assuming instability of the product.

8-Amino-isobutylflavin 5



4 **5**

A round-bottomed-flask was charged with the unpurified starting material *N*-isobutyl-4-methylbenzene-1,3-diamine **4** (3.11 g) dissolved in glacial acetic acid (150 ml) and violuric acid monohydrate (3.06 g, 17.48 mmol, 1 eq.). The flask was flushed once with nitrogen before the stirred reaction mixture was heated to reflux for 45 minutes. The violuric acid dissolved rapidly in the heat. At the boiling point a yellow/orange solid started precipitating. The mixture was left at room temperature over the weekend. Then the orange precipitate was collected by suction filtration and washed with water (60 ml) and diethylether (20 ml). The desired product 8-Amino-isobutylflavin **5** (3.05 g, 10.2 mmol, 58 %) was obtained in moderate yield, considering the impure starting material.

Melting Point > 300°C

^{1}H NMR analysis: δ_H(200.13 MHz, $CDCl_3$)/ppm 10.94 (s, 1 H, NH), 7.61 (d, 1 H, C6H), 7.20 (bs, 2 H, NH_2), 6.75 (s, 1 H, C9H), 4.31 (bs, 2 H, CH_2), 2.38 – 2.20 (m, 1 H, CH_3*CH*CH_3), 2.21 (s, 3 H, C_{ar}-*CH_3*), 0.93 (d, 6 H, *CH_3*CH*CH_3*).

^{13}C NMR analysis: δ_C(50.3 MHz, $CDCl_3$)/ppm 160.9, 155.8, 155.7, 150.9, 134.9, 133.0 (CH_{ar}), 130.1, 127.7, 124.9, 94.8 (CH_{ar}), 50.0 (CH_2), 26.0 (*CH*-$(CH_3)_2$), 19.8 (*CH_3*CH*CH_3*), 16.8 (C_{ar}-*CH_3*).

ν_{max}(KBr)/cm^{-1} 3427*m* and 3340*m* and 3302*m* (NH), 3219*s*, 3007*m* (CH_{ar}), 2960*m* and 2821*m* (CH_{alk}), 1695*s*, 1667*s*, 1636*s*, 1520*s*, 1454*s*, 1399*m*, 1324*s*, 1267*s*, 1247*s*, 1209*s*, 1012*m*, 871*m*, 846*m*, 743*m*, 620*m*.

m/z (ESI/MS) 322 (M+Na), 300 (M^+).

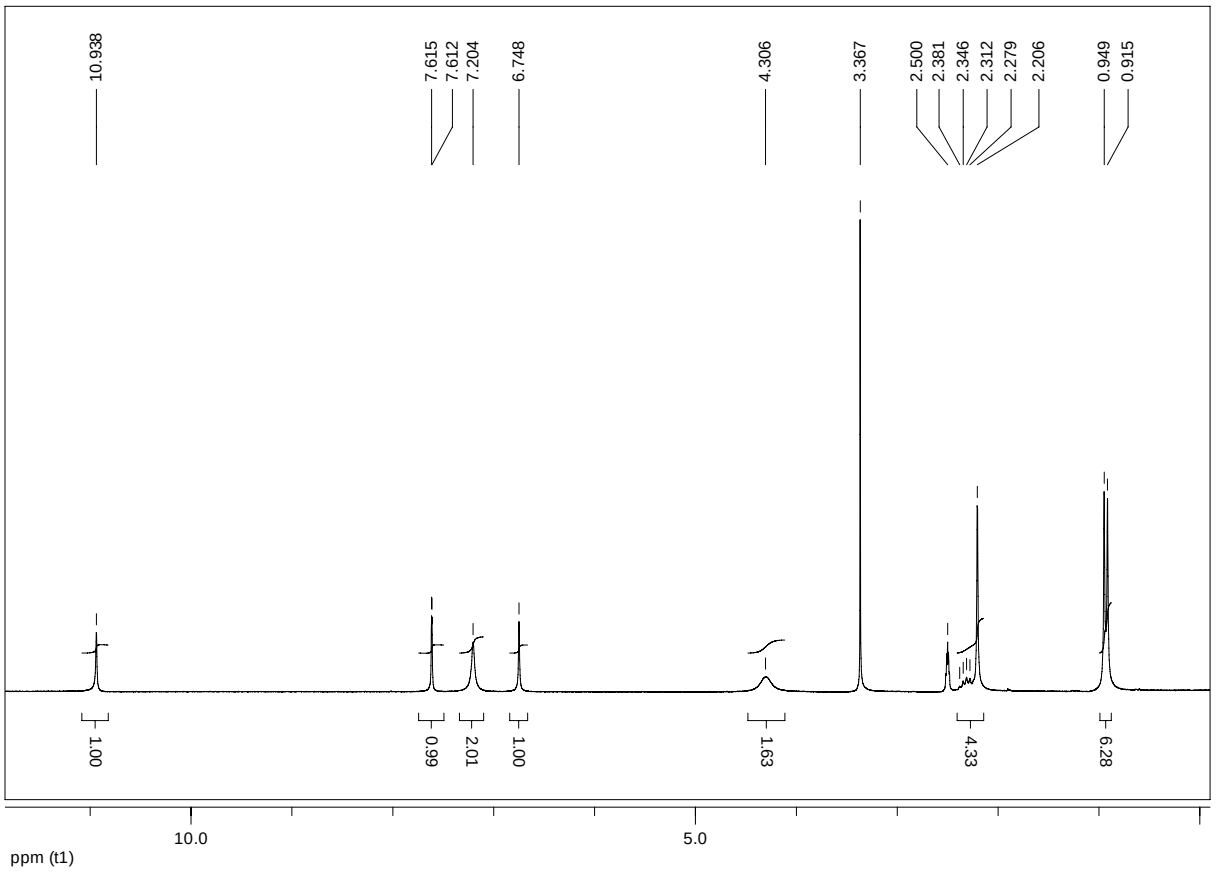


Fig. S5 ^{1}H NMR spectra of 8-Amino-isobutylflavin **5**

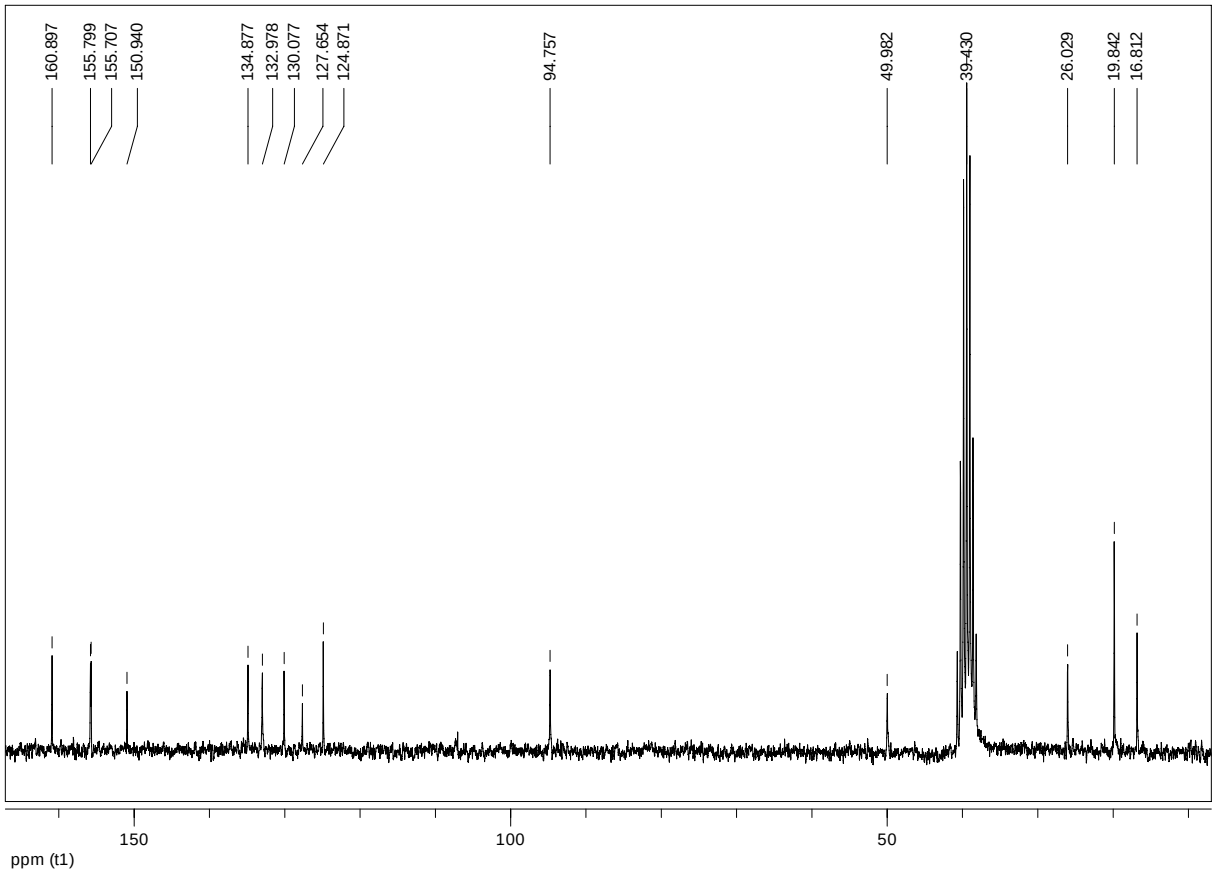
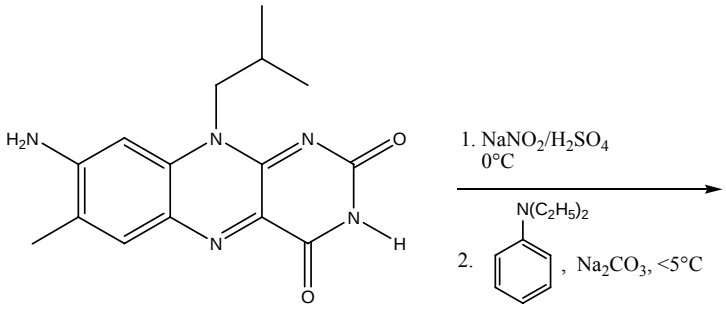
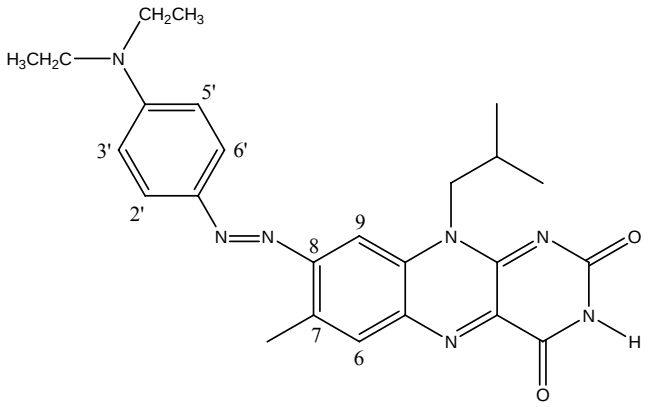


Fig. S6 ^{13}C NMR spectra of 8-Amino-isobutylflavin **5**

8-[[*p*-bis(ethyl)amino]phenyl]azo]-isobutylflavin 6 (ABFL)



5



6

The diazonium salt solution **A** of 8-amino-isobutylflavin **5** was prepared as follows:

5 (295 mg, 0.99 mmol, 1 eq.) was dissolved in glacial acetic acid (35 ml) in the heat. This solution was allowed to cool down to room temperature again. On dropwise addition of the flavin-solution to a stirring mixture of sodium nitrite (75 mg, 1.09 mmol, 1.1 eq.) and conc. sulphuric acid (1.5 ml), prepared at 0°C, a colour change from colourless over red to yellow occurred. It is essential to maintain the temperature of the reaction mixture below 5°C. To prevent the mixture from freezing it might be necessary to add some water to lower the melting point. The diazonium salt solution was stirred for another 30 minutes at 0°C.

To form the diazo-linkage a round bottomed flask was charged with *N*,*N*-diethylaniline and aqueous sulphuric acid (18 wt%, 5 ml) and cooled down to 0°C. The diazonium salt solution **A** was afterwards added dropwise to the acidic aniline mixture. Instantly a colourchange to blue-green (sometimes purple) took place. Subsequently the pH of the reaction mixture was raised to 4.2 by careful addition of solid sodium carbonate. At times water was added to prevent high viscosity of the mixture. All work should be done at temperatures below 5°C. On raising the pH a bluish-green precipitate formed. This was collected by filtration after another 4 hours stirring at 0°C and washed intensively with water to remove sodium carbonate.

The crude product 8-[[*p*-bis(ethyl)amino]phenyl]azo]-isobutylflavin **6** (244 mg, 0.53 mmol, 54 %) has got a glittering greenish colour. TLC analysis showed only little impurities. For further purification recrystallisation of 100 mg of crude product was carried out in a boiling mixture of ethanol and acetone (5 : 1), yielding 85 mg pure product useable for further analysis.

Melting Point: not measurable because of decomposition.

^{1}H NMR analysis: δ_H(200.13 MHz, $CDCl_3$)/ppm 8.38 (bs, 1 H, NH), 8.17 (d, 1 H, C6H), 7.92 (d, 2 H, C2'H, C6'H), 7.75 (s, 1 H, C9H), 6.76 (d, 2 H, C3'H, C5'H), 4.67 (bs, 2 H, CH_2), 3.52 (quart., 4 H, 2 x CH_2CH_3), 2.77 (s, 3 H, C_{ar}-CH_3), 2.57 – 2.38 (m, 1 H, CH_3CHCH_3), 1.28 (t, 6 H, 2 x CH_2CH_3), 1.06 (d, 6 H, CH_3CHCH_3).

ν_{max}(KBr)/cm^{-1} 3468*bw*, 3149*w*, 3083*w* (CH_{ar}), 2959*w* (CH_{alk}), 2818*w* (CH_{alk}), 1713*m*, 1655*m*, 1601*m*, 1571*m*, 1530*s*, 1376*m*, 1346*m*, 1275*m*, 1253*m*, 1208*m*, 1155*m*, 1116*m*, 1077*m*, 1006*m*, 854*w*, 708*w*, 545*w*.

m/z (EI/MS) 459 (M^+), 403 (M^+-C_4H_9), 388 (M^+-$C_4H_{10}N$), 360, 243.

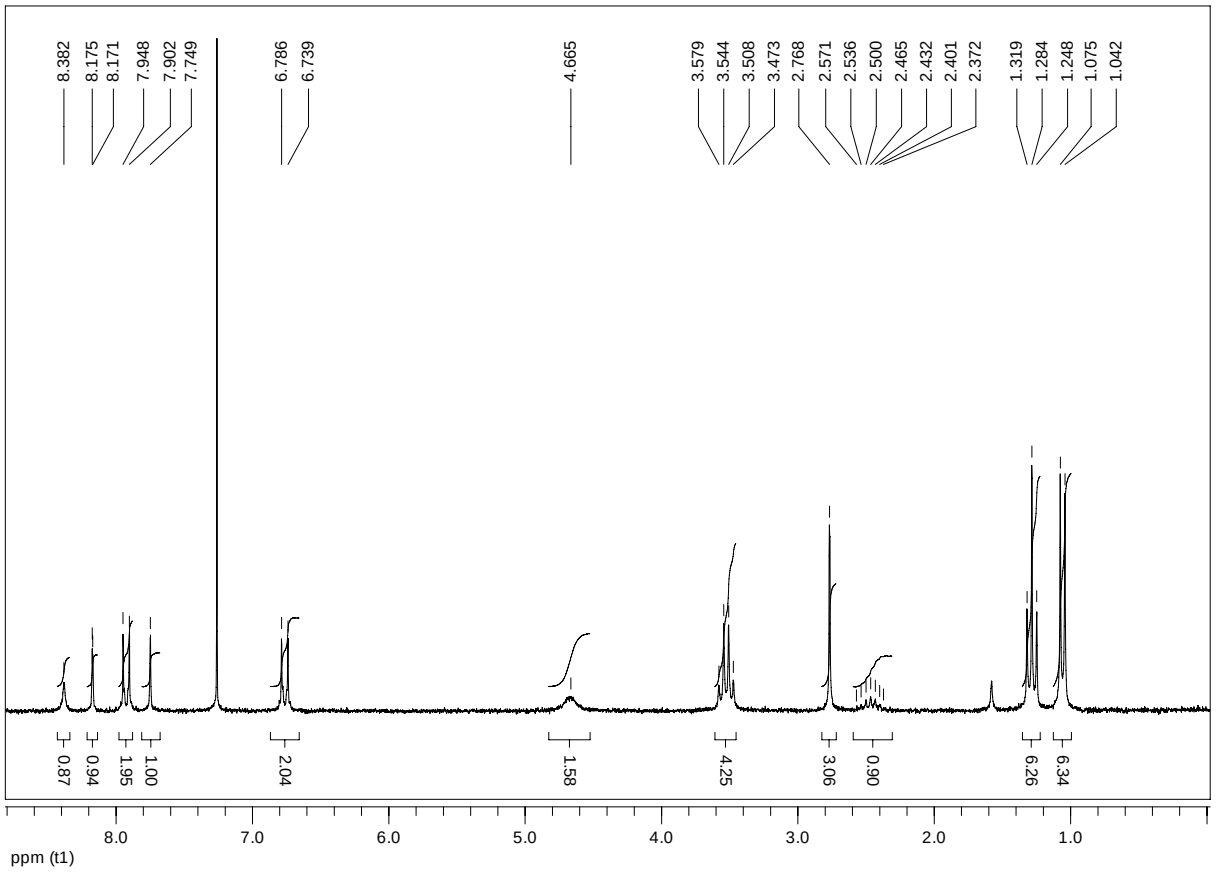
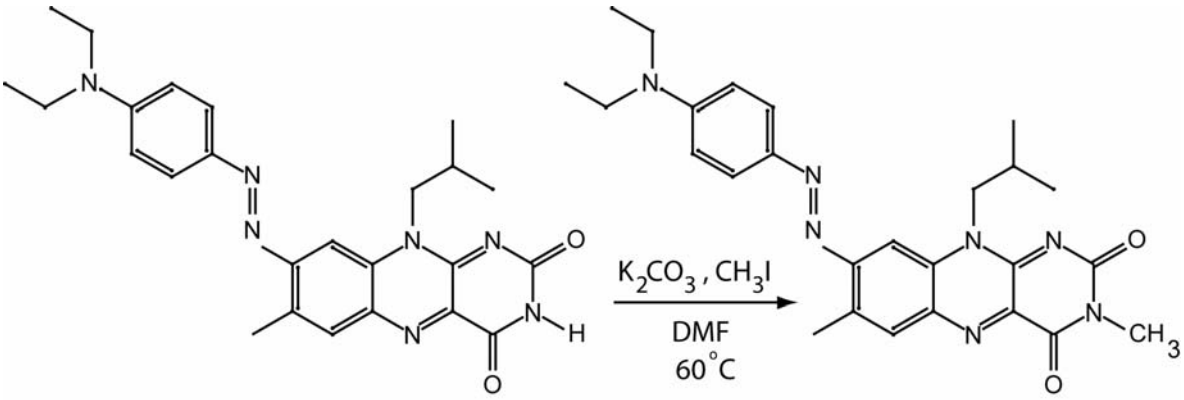


Fig. S7 ^{1}H NMR spectra of 8-[[*p*-bis(ethyl)amino]phenyl]azo]-isobutylflavin **6**

8-[[*p*-bis(ethyl)amino]phenyl]azo]-3-methyl-isobutylflavin 7 (MABFL)



To a solution of 8-[[*p*-bis(ethyl)amino]phenyl]azo]-isobutylflavin **6** (0.13 mmol) and K_2CO_3 (0.52 mmol) in DMF (5 ml) at 60°C was added CH_3I (0.26 mmol) and the reaction was allowed to proceed overnight. Upon cooling, the solvent was reduced under reverse pressure. The reaction was quenched with water and extracted with chloroform. The organic layers were combined, washed extensively with water and then washed with brine, anhydrous Na_2SO_4, filtered, and reduced under reverse pressure onto silica gel. The crude product was then purified using flash column chromatography (1:1 Hexane/Ethyl Acetate) followed by recrystalization from methanol yielding 8-[[*p*-bis(ethyl)amino]phenyl]azo]-3-methyl-isobutylflavin **7** (71% yield).

Melting Point: not measurable because of decomposition.

^{1}H NMR analysis: δ_H(400.13 MHz, $CDCl_3$)/ppm 8.19 (d, 1 H, C6H), 7.92 (d, 2 H, C2'H, C6'H), 7.75 (s, 1 H, C9H), 6.76 (d, 2 H, C3'H, C5'H), 4.67 (bs, 2 H, CH_2), 3.53 (s, 3H, CH_3), 3.52 (quart., 4 H, 2 x *CH_2*CH_3), 2.77 (s, 3 H, C_{ar}-*CH_3*), 2.47 – 2.42 (m, 1 H, CH_3*CH*CH_3), 1.28 (t, 6 H, *J* 7.1 Hz, 2 x CH_2*CH_3*), 1.06 (d, 6 H, *J* 6.7 Hz, *CH_3*CH*CH_3*)

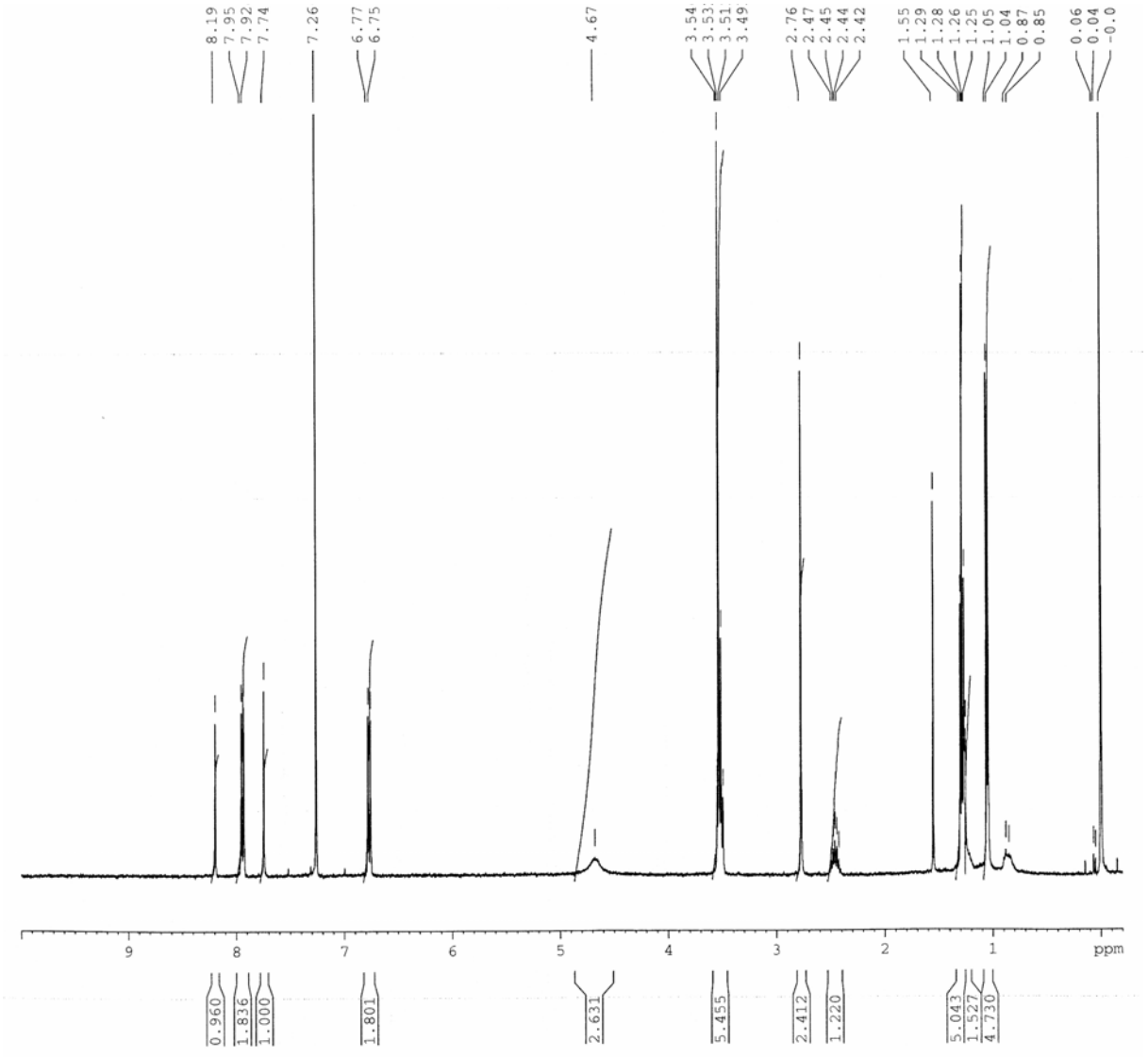
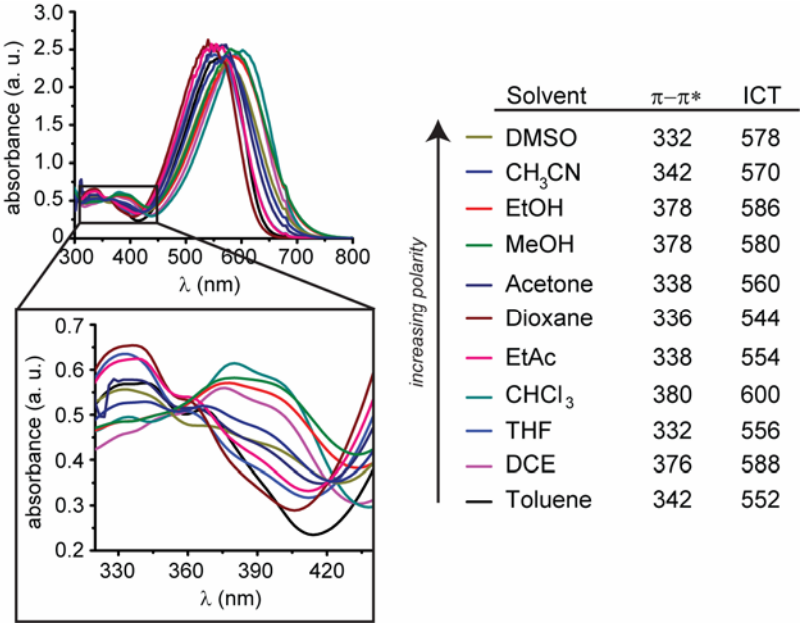


Fig. S8 ^{1}H NMR spectra of 8-[[*p*-bis(ethyl)amino]phenyl]azo]-3-methyl-isobutylflavin **7**

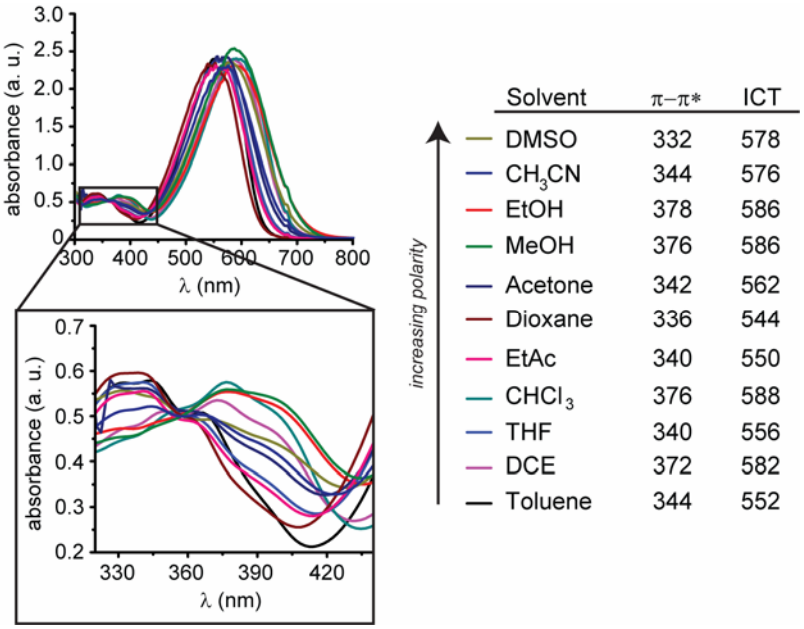
UV-Vis spectroscopy

All measurements were performed on HP 8452A Diode Array Spectrophotometer using a quartz crystal cuvette with 1 cm pathlength. Stock solutions (125 uM) of both **ABFL** and **MABFL** were diluted to specific concentrations to optimize the signal within the UV-Vis spectra. Comparison of eleven different solvent with various degrees of polarity were performed at 62.5 uM concentration for both **ABFL** and **MABFL**. The spectra are provided below with a table to highlight both the π-π* transition and intramolcular charge transfer (ICT) regions.



Solvent	π–π*	ICT
DMSO	332	578
CH_3CN	342	570
EtOH	378	586
MeOH	378	580
Acetone	338	560
Dioxane	336	544
EtAc	338	554
$CHCl_3$	380	600
THF	332	556
DCE	376	588
Toluene	342	552

Fig. S9 ABFL in eleven different solvents at 62.5 uM concentration



Solvent	π–π*	ICT
DMSO	332	578
CH_3CN	344	576
EtOH	378	586
MeOH	376	586
Acetone	342	562
Dioxane	336	544
EtAc	340	550
$CHCl_3$	376	588
THF	340	556
DCE	372	582
Toluene	344	552

Fig. S10 MABFL in eleven different solvents at 62.5 uM concentration

Samples were further diluted to the following concentrations (31.25 uM, 15 uM, 8 uM, 4 uM, 1 uM) and measured using UV-Vis. The concenations were plotted versus the charge transfer absorbance intensity (at the specifc λ_{max} for each solvent) to determine the type of charge transfer process that occured for both **ABFL** and **MABFL**.

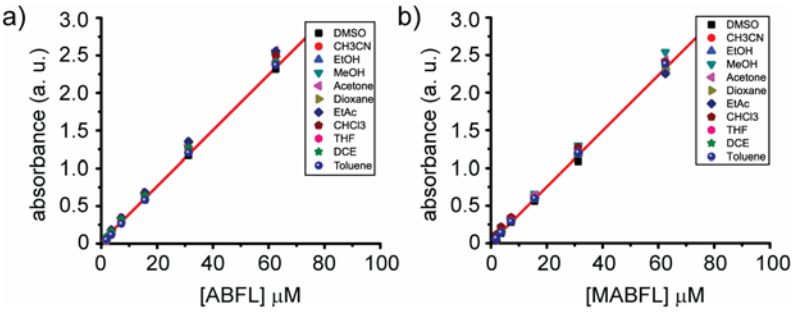


Fig. S11 Both **ABFL** and **MABFL** demonstrate linear dependence between concentration and absorbance intensity indicating an ICT process

The binding constant for the [**ABFL:DAP**] complex was calculated from the **ABFL/DAP** UV-Vis titration. Aliquots of **DAP** (1.5 mM) guest were titrated into an **ABFL** (15 uM) host solution. The absorbance intensity at λ_{max} = 374 nm was plotted versus concentration of **DAP**. Data was fit to a binding isotherm using a nonlinear least square fit.

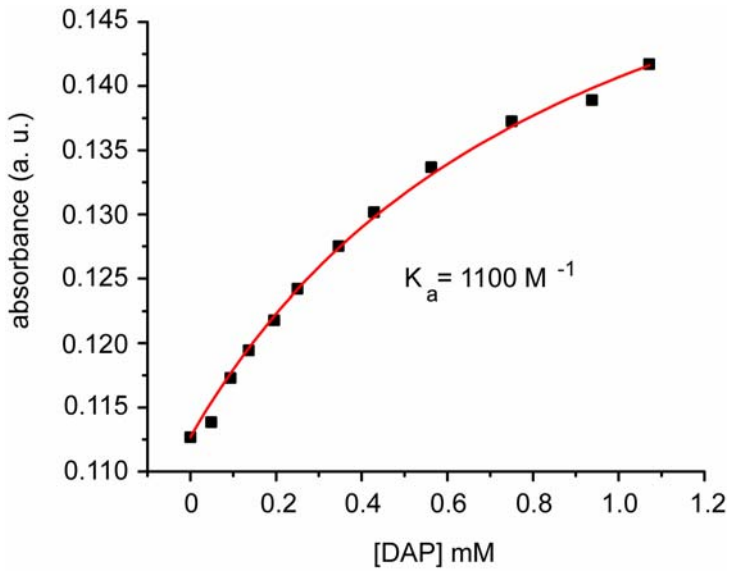


Fig. S12 Plot of **ABFL** absorbance intensity versus **DAP** concentration

Methylated **DAP** (at amide postions) was synthesized using a previous published procedure (A. L. Moraczewski, L. A. Banaszynski, A. M. From, C. E. White, and B. D. Smith, *J. Org. Chem.,* 1998, **63**, 7258.) Titration of methylated **DAP** (1.5 mM) into **ABFL** (15 uM) was used to assess the hydrogen bonding of **ABFL** with a non-complementary **DAP** derivative. No shift was apparent in the spectrum suggesting that there was no hydrogen bonding between **ABFL** and the methylated **DAP** derivative. Only titration endpoints were necessary to demonstrate that **ABFL** remains unchanged upon addition of methylated **DAP**. Spectra were corrected for any overlapping absorbance from methylated **DAP**.

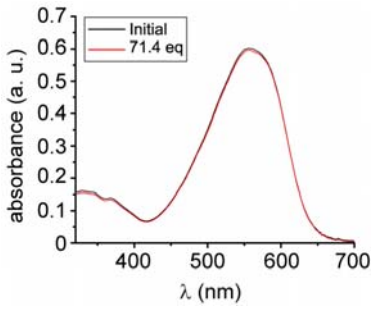


Fig. S13 Plot of **ABFL** spectrum at initial (no methylated **DAP**) and final (excess methylated **DAP**) titration endpoints.

Kamlet-Taft parameters

All parameters were taken from the following reference:

A. F. Lagalante, R. J. Jacobson, and T. J. Bruno, *J.Org. Chem.*, 1996, **61**, 6404.

Solvent	π*	α	β
DMSO	1	0	0.76
CH_3CN	0.85	0.15	0.4
EtOH	0.54	0.86	0.75
MeOH	0.6	0.98	0.66
Acetone	0.71	0.08	0.43
Dioxane	0.55	0	0.37
EtAc	0.55	0	0.45
$CHCl_3$	0.58	0.2	0.1
THF	0.58	0	0.55
DCE	0.81	0	0.1
Toluene	0.54	0	0.12

Blue = primarily hydrogen bond donating
Red = primarily hydrogen bond accepting

Fig. S14 Kamlet-Taft parameters in terms of polarizability, π*, hydrogen bond donation, α, and hydrogen bond acceptance, β.