Supplementary Materials

Safranine O was purchased from Aldrich. All reactions were carried out under an inert atmosphere. Commercial reagents were used without further purification unless otherwise noted. For analytical thin-layer chromatography, precoated glass silica gel plates (EMD silicagel 60 F_{254}) were used. All products were purified using silica gel (Analtech 30-75 μm) and/or by recrystallization. Melting points are uncorrected and were determined using a Fisher-Johns melting point apparatus. NMR spectra were recorded in $CDCl_3$ with TMS as an internal standard at room temperature on a Varian Inova operating at 500 MHz for ^1H, 125 MHz for ^{13}C and 50 MHz for ^{15}N. When necessary, two-dimensional homonuclear correlation (COSY) spectra were run in order to confirm assignments.

N-tert-butoxycarbonyl-5-iodo-2-methylaniline (2). A solution of 5-iodo-2-methylaniline (1g, 42.91mmol) and 2-tert butyldicarbonate (1.03g, 47.19 mmol) in THF (10 ml) was stirred at reflux temperature for 2 hours. Solvent was then removed in vacuo, the residue dissolved in ethyl acetate and this solution was washed successively with 1M citric acid solution and brine. The organic phase was dried over $MgSO_4$ and concentrated in vacuo. The resultant yellow-white solid was crystallized from hexane to give white solid **2** (1.22 g, 85.3%). mp 119° C (hexane); R_f 0.8 (3:1, hexane/ethylacetate); λ_{abs}max ($CDCl_3$) 246 nm; ESIMS: *m/z* 356.1 (M^++1), 372.1(M^++K). ^{1}H-NMR ($CDCl_3$,500MHz): δ 1.52 [9H, s, C(C*H*$_3$)$_3$], 2.18 [3H, s, C*H*$_3$), 6.21 [1H, bs, **o**-Ph*H*], 6.84 [1H, d, *J* = 7.1 Hz, **m**-Ph*H*], 7.30 [1H, dd, *J* = 7.8, 1.5 Hz, **p**-Ph*H*], 8.25 [1H, bs, N*H*]. ^{13}C-NMR ($CDCl_3$, 125MHz): δ 17.67 [*C*H$_3$], 28.44 [C(*C*H$_3$)$_3$], 81.14 [*C*(CH$_3$)], 128.88 [*C*(5)], 131.85 [*C*(4)], 132.10 [*C*(2)], 132.41 [*C*(1)], 132.56 [*C*(6)], 137.83 [*C*(3)], 152.73 [NH*C*O].

Tert-butyl 5,5'-(4-methoxy phenylazanediyl) bis (2-methyl-5,1-phenylene)dicarbamate (4). P-anisidine **3** (300 mg, 2.44 mmol) was added to a solution containing **2** (1.7 g, 5.11 mmol), KO_t-Bu (819.76mg, 7.31mmol), CuI (18.5 mg, 0.097 mmol) and 2,2'-bipyridine (15.2 mg, 0.097 mmol) in dry toluene (7ml) and then stirred at 120° C for 4h. The reaction was filtered and concentrated in vacuo. The crude product was purified by flash chromatography [4:1 hexane/ethylacetate, 1% triethylamine] resulting in 1.01g (78.3%) of a golden yellow oil. R_f 0.27 (4:1, hexane/ethylacetate, 1% triethylamine); λ_{abs}max ($CDCl_3$) 304 nm, ESIMS: *m/z* 534.3 (M^++1), 556.2(M^++Na). ^{1}H-NMR ($CDCl_3$,500MHz): δ 1.43 [9H, s, C(C*H*$_3$)$_3$], 2.17 [3H, s, C*H*$_3$], 3.78 [3H, s, OC*H*$_3$], 6.12 [2H, bs, C(4)*H*], 6.66 [1H, d, *J* = 2.4 Hz, **o**-Methoxy*Ph*], 6.67 [1H, d, *J* = 2.0 Hz, **m**-Methoxy*Ph*], 6.95 [2H, d, *J* = 8.2 Hz, C(6)*H*], 7.04 [2H, d, *J* = 8.2 Hz, C(3)*H*], 7.35 [2H, bs, N*H*].^{13}C-NMR ($CDCl_3$, 125MHz): δ 17.44 [*C*H$_3$], 28.50 [C(*C*H$_3$)$_3$], 55.67 [O*C*H$_3$], 81.5 [*C*(CH$_3$)],114.81 [*C*(6)] , 117.07 [**m**-Methoxy*Ph*], 119.38 [*C*(4)] , 122.18 [*C*(2)], 126.85 [*C*(3)], 130.92 [**o**-Methoxy*Ph*], 136.86 [*C*(1)], 141.20 [*C*(5)], 147.10 [diPhN*C*-MethoxyPh], 153.20 [*C*=O], 155.89 [**p**- Methoxy*Ph*].

2,2'-(5,5'-(4-methoxyphenylazanediyl)bis(2-methyl-5,1-phenylene))diisoindoline-1,3-dione (5). Deprotection and phthaloylation of the amino groups in **4** was performed in a single step. 2.1 equivalents of phthalic anhydride (932.7 mg, 6.29 mmol) is added to **4** (1 g, 2.99 mmol) and the reactants stirred in 15 ml of glacial acetic acid at119° C for 4h. The resultant dark brown colored solution was subsequently washed with saturated sodium bicarbonate and then brine, dried over $MgSO_4$, filtered and concentrated in vacuo. The resultant yellow oil was purified using flash chromatography [1:1 hexane/ethylacetate, 1% triethylamine] resulting in 1.5g, (86%) of a light yellow oil. R_f 0.7 (1:1, hexane/ethylacetate, 1% triethylamine); λ_{abs}max ($CDCl_3$) 302 nm; ESIMS : m/z 594.4 (M++1), 616.4 (M++Na); ^{1}H-NMR

($CDCl_3$,500MHz): δ 2.11 [6H, s, CH3] , 3.76 [3H ,s, OCH3], 6.82[1H, bs, C(4/4')H], 6.84 [1H, bs, C(4/4')H], 6.92[2H, d, J = 2.0 Hz, C(3/3')H], 7.1 [1H, d, J = 2.5 Hz, C(6/6')H], 7.11 [1H, d, J = 2.4 Hz, C(6/6')H], 7.15 [2H, d, J = 8.6 Hz, o-MethoxyPh], 7.19 [2H, d, J = 8. 3 Hz, m-MethoxyPh], 7.76 [4H, dd, J = 2.9, 5.38 Hz, αH-Isoind], 7.91[4H, dd, J = 2.9, 5.37 Hz, βH-Isoind]. ^{13}C-NMR ($CDCl_3$, 125MHz): δ 17.58 [CH3], 55.67 [OCH3], 115.61 [C(6)] , 122.96 [m-MethoxyPh], 123.70 [C(4)], 123.94 [αC-Isoind], 127.67 [C(2)], 129.99 [C(3)], 131.37 [4C-Isoind], 131.84 [o-MethoxyPh], 132.27 [βC-Isoind], 134.48 [C(1)], 140.04 [C(5)], 146.65 [diPhN-C-MethoxyPh], 156.68 [p- MethoxyPh], 167.45 [1,3 C dione].

2,8-bis(1,3-dioxoisoindolin-2-yl)-10-(4-methoxyphenyl)-3,7-dimethylphenazine-10-ium 5-oxide (6). **5** (100 mg, 0.17 mmol) was dissolved in 17 ml acetic acid and 3 ml of dry THF and the solution cooled to 0° C with ice. ^{15}N labeled sodium nitrite (60 mg, 0.85 mmol) was added all at once, the ice was removed, and the solution was stirred while warming to room room temperature for 3 h. After completion of the reaction as monitored by TLC the resultant deep red solution was washed with saturated sodium bicarbonate solution and then brine, dried over $MgSO_4$, filtered and concentrated in vacuo.. The resultant dark red oil was purified using flash chromatography [1:1 hexane/ethylacetate, 1% triethylamine] resulting in 57.7 mg (55%) of a sticky orange solid. R_f 0.4 (1:1, hexane/ethylacetate, 1% triethylamine); λ_{abs}max ($CDCl_3$) 450 nm; ESIMS: m/z 623.3 (M++1), 640.3 (M++H2O);^{1}H-NMR ($CDCl_3$,500MHz): δ 2.12 [6H, s, CH3], 3.83 [3H, s, OCH3], 6.84 [1H, d, J = 2.4 Hz, C(4/6)H], 6.98 [1H, d, J = 2.4 Hz, C(4/6)H], 7.01[1H, d, J = 2.4 Hz, C(1/9)H], 7.03[1H, dd, J = 2.9, 8.8 Hz, C(1/9)H], 7.14 [2H, d, J = 8.3 Hz, m-MethoxyPh], 7.26 [1H, dd, J = 0.98, 8.8 Hz, o-MethoxyPh], 7.28 [1H, dd, J = 2.9, 5.9 Hz, o-MethoxyPh], 7.67 [1H, dd, J = 3.4, 5.37 Hz, αH-Isoind], 7.82 [1H, dd, J = 2.9, 5.4 Hz, βH-Isoind]; ^{13}C-NMR ($CDCl_3$, 125MHz): δ 17.89 [CH3], 56.4 [OCH3], 110.51 [m-MethoxyPh], 122.51 [C(1)], 123.48 [αC-Isoind],124.14 [C(5)], 131.28 [C(6)], 131.75 [o-MethoxyPh], 132.20 [C(4)], 132.41 [4C-Isoind], 132.62 [βC-Isoind], 133.06 [C(3)], 134.68 [C(2)], 145.27 [diPhN-C-MethoxyPh], 157.49 [p-MethoxyPh], 167.44 [1,3 C dione]; ^{15}N-NMR ($CDCl_3$, 50 MHz): δ 371.49

3,7-diamino-5-(4-methoxyphenyl)-2,8-dimethylphenazin-5-ium (7). **6** (10 mg, 0.016 mmol) was dissolved in isopropanol (10 ml) cooled to 0° C. $NaBH_4$ (3.03 mg, 0.08 mmol) was added and the solution stirred for 24 h at room temperature. When TLC indicated that **6** had been completely consumed, glacial acetic acid was added dropwise until fuming subsided. The flask was then fitted with a condenser and heated to 80° C for 2h. The crude solution was filtered, concentrated in vacuo and then lyophilized in the dark for 48 h to remove trace amounts of acetic acid. The reaction mixture was purified by flash chromatography [6:3:1 isopropanol/ethylacetate /5% aqueous acetic acid] yielding a purple powder (2.2 mg, 40%). R_f 0.3 (6:3:1 isopropanol/ethylacetate /5% aqueous acetic acid). Further purification was done by HPLC as described below. λ_{abs}max (20 mM KH_2PO_4, 100 mM KCl, pH=8) 538nm; λ_{em}max (20 mM KH_2PO_4, 100 mM KCl, pH=8) 623nm; ESIMS: m/z 347.2 (M++1); ^{1}H-NMR ($CDCl_3$,500MHz): δ 2.23[6H, s, CH3], 3.94 [3H, s, OCH3], 6.56 [2H, bd, C(1/9)H], 6.58[2H, bd, C(1/9)H], 6.92 [2H, bd, C(4/6)H], 6.94[2H, bd, C(4/6)H], 7.53[4H, dd, J = 3.4, 5.86 Hz, m-MethoxyPh], 7.63[4H, dd, J = 3.4, 5.9 Hz, o-MethoxyPh]; ^{15}N-NMR ($CDCl_3$, 50 MHz): δ 380.66.

HPLC purification of safranines

Final purifications of both safranine O and its methoxy derivative were performed using a Shimadzu HPLC equipped with two LC-6AD pumps and a multiwavelength photodiode array detector with a scanning range of 200-800 nm. Preparative scale purification was performed at room temperature using a 250 x 20 mm 10μm Proto 300 C18 column (Higgins Analytical)at a flow rate of 12 ml/min. Buffer A

consisted of 9:1-water/methanol (v/v) and 5% phosphoric acid, pH 2. Buffer B consisted of methanol. For safranine O, the applied gradient was from 48 to 72% of Buffer B over 20 min. For the methoxy derivative, the gradient was from 42.5 to 60% Buffer B over 20 min. Retention times were 11.3 min for Safrinine O and 12.5 min for the methoxy derivative.