Discovery of Fluorescent Cyanopyridine and Deazalumazine Dyes using Small Molecule Macroarrays

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General experimental information.

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AC-300 spectrometer in deuterated solvents at 300 MHz and 75 Hz, respectively. Chemical shifts are reported in parts per million (ppm, δ) using tetramethyl silane (TMS) as a reference (0.0 ppm). Couplings are reported in hertz. LC-MS (ESI) were obtained using a Shimadzu LCMS-2010 (Columbia, MD) equipped with two pumps (LC-10ADvp), controller (SCL-10Avp), autoinjector (SIL-10ADvp), UV diode array detector (SPD-M10Avp), and single quadrupole analyzer (by electrospray ionization, ESI). The LC-MS is interfaced with a PC running the Shimadzu LCMSsolution software package (Version 2.04 Su2-H2). A Supelco (Bellefonte, PA) 15 cm × 2.1 mm C-18 wide-pore reverse phase column was used for all LC-MS work. Standard reverse phase HPLC conditions were as follows: flow rate = 200 μL/min; mobile phase A = 0.4% formic acid; mobile phase B = 0.2% formic acid in acetonitrile. Attenuated total reflectance (ATR)-IR spectra were recorded with a Bruker Tensor 27 spectrometer, outfitted with a single reflection MIRacle Horizontal ATR by Pike Technologies. A ZnSe crystal with spectral range 20,000 to 650 cm^{-1} was used. UV spectra were recorded using a Cary 50 Scan UV-Vis spectrometer running Cary WinUV 3.00 software. Fluorescence spectra were recorded on a Hitachi F-4500 Fluorescence spectrophotometer using the following parameters: 5.0 nm excitation slit width, 5.0 nm emission slit width, 1200 nm/min scan speed, 700 V PMT voltage, and 2.0 sec response time. Quantum yields were calculated using standard methods.[1] Thin layer chromatography (TLC) was performed on silica gel 60 F_{254} plates (E-5715-7, Merck). Silica gel 60 (230-400 mesh, EM Science) was used for flash column chromatography.[2] All reported melting points are uncorrected.

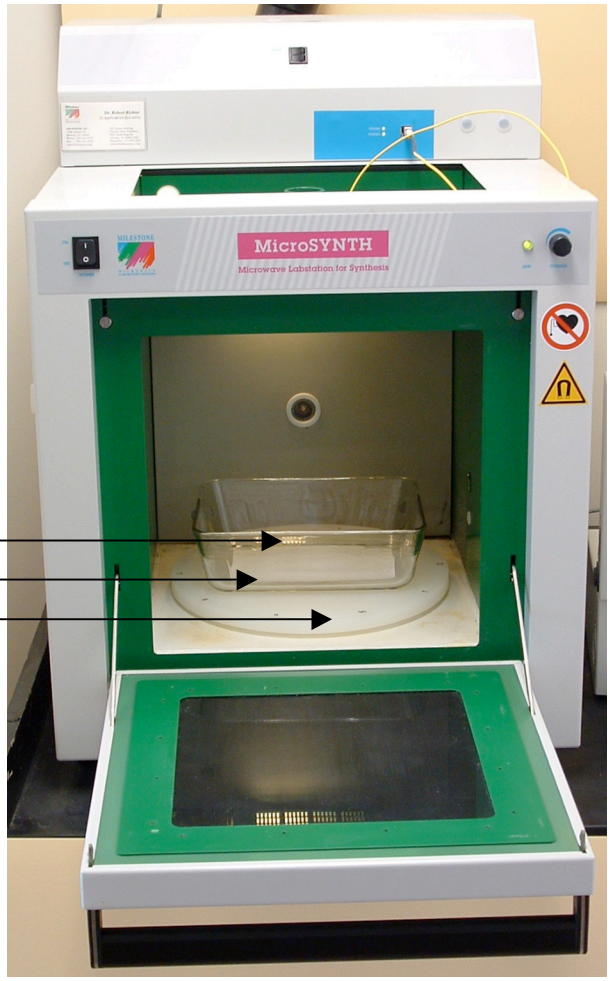
All reagents were purchased from commercial sources (Alfa-Aesar, Aldrich, and Acros) and used without further purification. Solvents were purchased from commercial sources (Aldrich and J.T. Baker) and used as is, with the exception of dichloromethane (CH_2Cl_2), which was distilled over calcium hydride immediately prior to use. Planar cellulose membranes (Whatman 1Chr chromatography paper, 20 x 20 cm squares) were purchased from Fischer Scientific and stored in a dessicator at room temperature until ready for use.

Microwave instrumentation. All microwave-assisted reactions were performed in a Milestone MicroSYNTH Labstation multimodal microwave synthesis reactor equipped with a continuous power source (1000 W max). This instrument is interfaced with an Ethos MicroSYNTH Lab Terminal PC running EasyWave reaction monitoring software. Using this reactor system, microwave irradiation can be applied to reactions using either power (wattage) control or temperature control. The microwave reactor is equipped with a fiber-optic temperature sensor that allows direct monitoring of the internal temperature of reaction vessels and an infrared sensor (installed in the side wall of the reactor cavity) that can monitor the surface temperature of reaction vessels inside the cavity.[3] Solvent depths of *ca*. 1 cm in the reaction vessel are required for accurate temperature monitoring using the submerged fiber-optic temperature probe. Solution-phase reactions were performed in the MicroSYNTH reactor using specialized 70 mL Teflon/PEEK reaction vessels. These vessels have appropriate holes in their lids to accommodate the fiber-optic temperature sensor in a protective ceramic sheath.

Planar cellulose supports were irradiated in a shallow Pyrex dish as depicted in the graphic on page S-3 on a rotating turntable inside the MicroSYNTH microwave reactor. Planar supports

were irradiated routinely using power control, as insufficient solvent volumes were used for accurate temperature monitoring using the fiber-optic probe.

Milestone MicroSYNTH Labstation



Solid-phase synthesis techniques. Planar cellulose membranes were mixed with reagents at room temperature and washed with solvents in glass vessels on a Lab-Line orbital shaker housed in a fume hood. All spotting procedures used in SPOT-synthesis[4] were performed manually using Brinkman Eppendorf pipettmen (calibrated for variable solvent delivery (1-10 μL)) and disposable polypropylene pipette tips.

Solution-phase synthesis of standard compounds (3e, 3f, 3n-p, 3q, 6c, and 6t).

Compound codes **C1**-**C10** are used for compounds not mentioned directly in the text.

1-(4-(tetrahydro-2*H*-pyran-2-yloxy)phenyl)ethanone (C1). 4-hydroxyacetophenone (8.5 g, 62.5 mmol), 3,4-dihydropyran (8.5 mL, 100 mmol), and *p*-toluenesulfonic acid (0.5 g 5.0 mmol) were dissolved in 125 mL of dichloromethane equipped with a stirbar and stirred for 17 h at room temperature. The reaction mixture was washed with saturated aq. Na_2CO_3, the organic layer was dried over $MgSO_4$, and concentrated under reduced pressure to afford 12.86 g of pale brown solid. 93% yield. Characterization matched that reported by Ma and Venanzi.[5]

4-dimethylamino-4'-(tetrahydro-2*H*-pyran-2-yloxy)chalcone (C2). Compound **C1** (2.64 g, 12.0 mmol), 4-dimethylaminobenzaldehyde (1.80 g, 12.0 mmol) were dissolved in EtOH (40 mL) in a 100 mL round bottom flask. A 2 mL aliquot of 50% (w/v) aqueous NaOH was added, and the solution was stirred overnight (approximately 18 h) at room temperature. The yellow precipitate was filtered and recrystallized from MeOH to afford 3.3 g of yellow-orange solid. 78% yield. TLC: R_f=0.31 (20% ethyl acetate in hexane); Melting point: 110-111 °C; ^{1}H-NMR (300 MHz, DMSO-d6) δ 8.09, 7.13 (AA'XX', $J_{AA'}$=$J_{XX'}$=2.4, J_{AX}=8.7, $J_{AX'}$=0.2 Hz, 4H), 7.63 (m, 2H), 7.61 (d, J=1.3 Hz), 6.72 (m, 2H), 5.6 (m, 1H), 3.73 (m, 1H), 3.59 (m, 1H), 3.02 (s, 6H), 1.80 (m, 3H), 1.57 (m, 3H); ^{13}C-NMR (75 MHz, DMSO-d6) δ 187.8, 160.7, 152.5, 145.0, 142.5, 131.2, 130.9, 122.8, 116.8, 116.6, 112.4, 96.22, 62.3, 30.3, 25.3, 19.1; IR (ATR): 2951, 1645, 1599, 1573, 1439, 1400, 1371, 1342, 1287, 1216, 1187, 1161, 1119 cm^{-1}; ESI-MS: expected, 351.2; observed, m/z 352.3 [M+H$^+$].

4'-hydroxy-4-methoxychalcone (C3). 4'-hydroxyacetophenone (1.63 g, 12 mmol) and *p*-anisaldehyde (1.45 mL, 12 mmol) were dissolved in MeOH (30 mL) in a 70 mL Teflon/PEEK Milestone microwave reaction vessel. A 2 mL aliquot of 50% (w/v) aqueous NaOH was added, and the solution was stirred until all reactants had dissolved. The reaction vessel was tightly closed and heated (with stirring) using a Milestone microwave reactor from room temperature to 150 °C over 15 min, held at 150 °C for 20 min, and allowed to cool to room temperature. The reaction mixture was poured over approximately 30 g of ice and acidified to pH 1.0 with 1M HCl, forming a yellow precipitate. The solid was isolated and recrystallized from MeOH to afford 0.90 g of golden crystals, 30% yield. TLC: R_f=0.25 (40% ethyl acetate in hexane); Melting point: 185-188 °C; ^{1}H-NMR (300 MHz, DMSO-d6) δ 10.35 (brs, 1H), 8.05, 6.89 (AA'XX', $J_{AA'}$=$J_{XX'}$=2.4, J_{AX}=8.6, $J_{AX'}$=0.2 Hz, 4H), 7.92, 7.00 (AA'XX', $J_{AA'}$=$J_{XX'}$=2.4, J_{AX}=8.6, $J_{AX'}$=0.2Hz, 4H), 7.80, 7.60 (AB peak, J= 15.5 Hz, 2H); ^{13}C-NMR (75 MHz, DMSO-d6) δ 187.7, 162.7, 161.8, 143.3, 131.7, 131.2m 130.0, 128.207, 120.3, 116.0, 115.0, 56.0; IR (ATR): 3200, 2990, 1643, 1602, 1562, 1512, 1430, 1352, 1286, 1223, 1165, 1046 cm^{-1}; ESI-MS: expected, 254.1; observed, m/z 254.8 [M+H$^+$].

4-fluoro-4'-hydroxychalcone (C4). 4'-hydroxyacetophenone (1.63 g, 12 mmol) and 4-fluorobenzaldehyde (1.28 mL, 12 mmol) were dissolved in 40 mL EtOH in a 100 mL round bottom flask. A 2 mL aliquot of 50% (w/v) aqueous NaOH was added, and the solution was stirred overnight (approximately 15.5 h) at room temperature. Approximately 30 g of ice were added to the solution, which was acidified to pH 4 with 1M HCl. The resulting pale yellow precipitate was isolated by filtration and recrystallized from a mixture of EtOH and water to afford 1.53 g of golden crystals. 54% yield. TLC: R_f=0.30 (20% ethyl acetate in hexane);

Melting point: 177-180 °C; ^{1}H-NMR (300 MHz, DMSO-d6) δ 10.39 (brs, 1H) 8.06, 6.90 (AA'XX', $J_{AA'}=J_{XX'}=2.4$, $J_{AX}=8.7$, $J_{AX'}=0.3$Hz, 4H), 7.93 (m, 2H), 7.95, 7.58 (AB peak, J= 15.6 Hz, 2H), 7.28(m, 2H); ^{13}C-NMR (75 MHz, DMSO-d6) δ 187.7, 163.9 (d, J=249.4 Hz) 162.9, 142.1, 132.2 (d, J= 2.8 Hz) 131.8, 131.6, (d, J = 9 Hz), 129.8, 122.7, 116.5 (d, J= 22 Hz), 116.0; IR (ATR): 3145, 1647, 1568, 1509, 1441, 1417, 1342, 1286, 1219, 1166, 1157 cm^{-1}; ESI-MS: expected, 242.1; observed, m/z 243.1 [M+H^{+}].

4,4'-dimethoxychalcone (C5). In a 100 mL round bottom flask, 4-methoxyacetophenone (1.5 g, 10.0 mmol), *p*-anisaldehyde (1.63 g, 12.0 mmol), 2.0 mL of 50% aq. NaOH, and 30 mL of MeOH were combined. The solution was closed with a stopper and stirred at room temperature for 8 h. The precipitate was filtered, washed with 2.0 mL of 50% aq. MeOH, and allowed to air dry. This procedure generated 2.29 g of **C5** as a white solid. 85% yield. TLC: R_f=0.50 (20% ethyl acetate in hexane); Melting point: 89-90 °C; ^{1}H-NMR (300 MHz, DMSO-d6) δ 8.16, 7.08 (AA'XX' peak, $J_{AA'}=J_{XX'}=2.4$, $J_{AX}=8.7$, $J_{AX'}=0.2$ Hz, 4H), 7.88, 7.63 (AB peak, J=15.3 Hz, 2H), 7.85, 7.02 (AA'XX' peak, $J_{AA'}=J_{XX'}=2.7$, $J_{AX}=8.7$, $J_{AX'}=0.2$ Hz, 4H), 3.86 (s, 3H), 3.82 (s, 3H); ^{13}C-NMR (75 MHz, DMSO-d6) δ 187.9, 163.7, 161.9, 143.8, 131.4, 131.3, 128.1, 120.2, 115.1, 114.6, 56.2, 56.0; IR (ATR): 3550, 3491, 1653, 1593, 1568, 1509, 1463, 1441, 1423, 1335, 1294, 1248, 1215, 1180, 1165, cm^{-1}; ESI-MS: expected, 268.1; observed, m/z 269.0 [M+H^{+}].

2-fluoro-4'-methoxychalcone (C6). Synthesized according to the above method for **C5**: 2.07 g; 65% yield; TLC: R_f=0.56 (20% ethyl acetate in hexane); Melting point: 92-93 °C; ^{1}H-NMR (300MHz, DMSO-d6) δ 8.17, 7.10 (AA'XX' peak, $J_{AA'}=J_{XX'}=2.4$, $J_{AX}=8.7$, $J_{AX'}=0.3$ Hz, 4H), 8.13 (m, 1H), 7.51 (m, 1H), 7.32 (m, 2H), 8.09, 7.72 (AB peak, J=15.7 Hz, 2H), 7.85, 7.02 (AA'XX' peak, $J_{AA'}=J_{XX'}=2.7$, $J_{AX}=8.7$, $J_{AX'}=0.2$ Hz, 4H), 3.86 (s, 3H), 3.82 (s, 3H); ^{13}C-NMR (75 MHz, DMSO-d6) δ 187.9, 164.1, 161.6 (d, J=255 Hz), 134.9 (d, J=4.5 Hz), 133.1 (d, J=8.0 Hz), 131.6 (d, J=7.4 Hz), 130.9, 129.7, 125.6, 124.9, 123.0 (d, J=14.6 Hz), 116.7 (d, J=23.3 Hz), 114.8, 56.2; IR (ATR): 2977, 1656, 1609, 1573, 1484, 1458, 1423, 1337, 1318, 1285, 1257, 1230, 1189, 1094 cm^{-1}; ESI-MS: expected, 256.1; observed, m/z 256.9 [M+H^{+}].

4-fluoro-4'-methoxychalcone (C7). Synthesized according to the above method for **C5**: 2.68 g; 87% yield; TLC: R_f=0.45 (40% ethyl acetate in hexane); Melting point: 107-108 °C; ^{1}H-NMR (300 MHz, DMSO-d6) δ 8.13, 7.05 (AA'XX' peak, $J_{AA'}=J_{XX'}=2.5$, $J_{AX}=8.7$, $J_{AX'}=0.3$ Hz, 4H), 7.93 (m, 2H), 7.87, 7.68 (AB peak, J=15.8 Hz, 2H), 7.26 (m, 2H), 3.81 (s, 3H); ^{13}C-NMR (75 MHz, DMSO-d6) δ 188.0, 164 (d, J=248 Hz), 163.9, 142.5, 132.2, 131.7 (d, J=8.6 Hz), 131.6, 131.1, 122.6, 116.5 (d, 22.3 Hz), 114.7, 56.2; IR (ATR): 3322, 1655, 1598, 1572, 1508, 1424, 1339, 1315, 1286, 1218, 1189, 1158 cm^{-1}; ESI-MS: expected, 256.1; observed, m/z 256.9 [M+H^{+}].

3',4',4-trimethoxychalcone (C8). Synthesized according to the above method for **C5**: 1.91 g; 64% yield; TLC: R_f=0.17 (20% ethyl acetate in hexane); Melting point: 73-74 °C; ^{1}H-NMR (300 MHz, DMSO-d6) δ 7.88 (dd, J=8.5, 2.0 Hz, 1H), 7.84, 7.01 (AA'XX' peak, $J_{AA'}=J_{XX'}=2.2$, $J_{AX}=8.9$, $J_{AX'}=-0.1$ Hz, 4H), 7.80, 7.69 (AB peak, J=15.5 Hz, 2H), 7.60 (d, J=2.0 Hz, 1H), 7.08 (d, J=8.5 Hz, 1H), 3.87 (s, 3H), 3.86 (s, 3H), 3.82 (s, 3H); ^{13}C-NMR (75 MHz, DMSO-d6) δ 187.9, 161.9, 153.7, 149.5, 143.7, 131.4, 131.3, 128.2, 123.8, 120.1, 115.0, 111.5, 111.4, 56.4, 56.3, 56.0; IR (ATR): 3383, 3200, 1649, 1594, 1568, 1513, 1459, 1424, 1336, 1295, 1265, 1248, 1201, 1180, 1163, 1150, 1055, 1027; ESI-MS: expected, 256.1; observed, m/z 256.9 [M+H^{+}].

3',4-dimethoxychalcone (C9). Synthesized according to the above method for **C5**: 1.54 g; 87% yield; TLC R_f=0.28 (20% ethyl acetate in hexane); Melting point: 45-47 °C; ^{1}H-NMR (300 MHz, DMSO-d6) δ 7.98 (d, J=15.7 Hz), 7.90 (dd, J=8.5, 1.8 Hz, 1H), 7.84 (d, J=8.4 Hz, 2H), 7.65 (d, J=15.7 Hz, 1H), 7.63 (d, J=8.5 Hz, 1H), 7.58 (d, J=1.8 Hz, 1H), 7.08 (d, J=8.4 Hz, 2H), 3.85 (s, 3H), 3.83 (s, 3H); ^{13}C-NMR (75 MHz, DMSO-d6) δ 187.8, 154.0, 149.5, 142.4, 134.8, 132.5, 131.4, 131.0, 124.4, 124.2, 123.4, 111.5, 111.3, 56.5, 56.2; IR (ATR): 2998, 2935, 2835, 1655, 1584, 1566, 1513, 1488, 1462, 1455, 1425, 1339, 1320, 1295, 1281, 1250, 1216 cm^{-1}; ESI-MS: expected, 268.1; observed, m/z 268.9 [M+H$^+$].

3-cyano-4,6-di(4-methoxyphenyl)-2-methylpyridine (3o). In a 250 mL round bottom flask equipped with a reflux condenser, 4,4'-dimethoxychalcone (**C5**, 1.07 g, 4.0 mmol), 3-aminocrotononitrile (328 mg, 4.0 mmol), NaOH (160 mg, 4.0 mmol), and 160 mL of EtOH were combined. The solution was refluxed for 8 h. The condenser was removed, and the solution was stirred at room temperature under an oxygen atmosphere. After 20 min, a precipitate formed. After 4 h, the precipitate was filtered, washed with 4.0 mL of 50% aq. MeOH, and allowed to air dry. This procedure generated 321 mg of **3o** as a white solid. 24% yield. TLC: R_f=0.56 (20% ethyl acetate in hexane); Melting point: 153-154 °C; ^{1}H-NMR (300 MHz, DMSO-d6) δ 8.19, 7.12 (AA'XX' peak, $J_{AA'}$=$J_{XX'}$=2.6, J_{AX}=8.4, $J_{AX'}$=0.3 Hz, 4H), 7.88 (s, 1H), 7.68, 7.05 (AA'XX' peak, $J_{AA'}$=$J_{XX'}$=2.6, J_{AX}=8.6, $J_{AX'}$=0.2 Hz, 4H), 3.83 (s, 3H), 3.82 (s, 3H), 2.76 (s, 3H); ^{13}C-NMR (75 MHz, DMSO-d6) δ 209.0, 162.6, 162.0, 161.4, 160.0, 158.4, 154.2, 130.9, 129.9, 129.1, 118.3, 116.9, 114.7, 56.0, 55.8, 24.5; IR (ATR): 2990, 2901, 2217, 1610, 1575, 1445, 1385, 1345, 1305, 1261, 1169, 1113, 1090, 1058 cm^{-1}; ESI-MS: expected, 330.1; observed, m/z 331.1 [M+H$^+$].

3-cyano-4-(2-fluorophenyl)-6-(4-methoxyphenyl)-2-methylpyridine (3n). Synthesized according to the above method for **3o**: 328 mg; 26% yield; TLC: R_f=0.60 (20% ethyl acetate in hexane); Melting point: 152-153 °C; ^{1}H-NMR (300 MHz, DMSO-d6) δ 8.20, 7.05 (AA'XX' peak, $J_{AA'}$=$J_{XX'}$=2.6, J_{AX}=8.7, $J_{AX'}$=0.3 Hz, 4H), 7.98 (s, 1H), 7.62 (m, 2H), 7.42 (m, 2H), 3.82 (s, 3H), 2.78 (s, 3H); ^{13C}-NMR (75 MHz, $CDCl_3$) δ 162.5, 161.9, 159.5 (d, J=249.9 Hz), 158.9, 148.3, 131.9 (d, J=8.2 Hz), 130.9 (d, J=2.3 Hz), 130.3, 129.2, 124.8 (d, J=3.9 Hz), 124.7, 118.1 (d, 2.2 Hz), 117.0, 116.8, 116.5, 144.6, 106.4, 55.6, 24.5; IR (ATR): 3344, 2990, 2901, 2217, 1610, 1585, 1539, 1488, 1444, 1386, 1309, 1261, 1220, 1171, 1066 cm^{-1}; ESI-MS: expected, 318.1; observed, m/z 319.0 [M+H$^+$].

3-cyano-4-(3,4-dimethoxyphenyl)-6-(4-methoxyphenyl)-2-methylpyridine (3q). Synthesized according to the above method for **3o**: 545 mg; 38% yield; TLC: R_f=0.20 (20% ethyl acetate in hexane); Melting point: 187-188 °C; ^{1}H-NMR (300 MHz, DMSO-d6) δ 7.95 (s, 1H), 7.86 (dd, J=8.5, 2.1 Hz, 1H), 7.80 (d, J=2.1 Hz), 7.70, 7.14 (AA'XX' peak, $J_{AA'}$=$J_{XX'}$=2.5, J_{AX}=8.6, $J_{AX'}$=0.2 Hz, 4H), 7.09 (d, J=8.6 Hz, 1H), 3.86 (s, 3H), 3.85 (s, 3H), 3.84 (s, 3H), 2.75 (s, 3H); ^{13}C-NMR (75 MHz, DMSO-d6) δ 162.4, 161.3, 158.4, 153.5, 151.7, 149.6, 130.9, 129.1, 121.5, 117.5, 115.0, 112.4, 111.2, 56.4, 56.3, 56.1, 24.8; IR (ATR): 2990, 2901, 2218, 1612, 1573, 1517, 1445, 1384, 1303, 1268, 1237, 1170, 1114, 1058 cm^{-1}; ESI-MS: expected, 360.2; observed, m/z 361.1 [M+H$^+$].

3-cyano-4-(3-methoxyphenyl)-6-(4-methoxyphenyl)-2-methylpyridine (3p). In a 250 mL round bottom flask equipped with a reflux condenser, 3',4-dimethoxychalcone (**C9**, 805 mg, 3.0

mmol), 3-aminocrotononitrile (246 mg, 3.0 mmol), sodium hydroxide (120 mg, 3.0 -mmol), and 120 mL of EtOH were combined. The solution was refluxed for 24 h. The condenser was removed, and the solution was stirred at room temperature under an oxygen atmosphere. After 48 h, the precipitate was filtered, washed with 5.0 mL of EtOH, and allowed to air dry. 321 mg of white solid were isolated. 32% yield. TLC R_f=0.33 (20% ethyl acetate in hexane); Melting point: 151-153 °C; ^{1}H-NMR (300 MHz, $CHCl_3$) δ 7.64 (m, 3H), 7.61, 7.05 (AA'XX' peak, $J_{AA'}$=$J_{XX'}$=2.5, J_{AX}=8.5, $J_{AX'}$=0.3 Hz, 4H), 7.41 (t, J=8.1 Hz, 1H), 7.01 (ddd, J=8.1, 2.7, 0.9 Hz, 1H), 3.90 (s, 3H), 3.89 (s, 3H), 2.90 (s, 3H); ^{13}C-NMR (75 MHz, $CHCl_3$) δ 163.0, 131.2, 160.3, 159.1, 153.7, 139.6, 130.2, 130.1, 128.9, 120.0, 118.1, 117.8, 116.2, 114.7, 113.0, 105.8, 55.7, 24.7; IR (ATR): 3031, 2959, 2845, 2221, 1609, 1602, 1582, 1569, 1541, 1514, 1493, 1475, 1445, 1426, 1383, 1368, 1328, 1310, 1296, 1257, 1235 cm^{-1}; ESI-MS: expected, 330.1; observed, m/z 330.9 [M+H$^+$].

3-cyano-4-(4-dimethylaminophenyl)-6-(4-(tetrahydro-2*H*-pyran-2-yloxy)phenyl)-2-methylpyridine (C10). In a 100 mL round bottom flask equipped with a reflux condenser, 4-dimethylamino-4'-(tetrahydro-2*H*-pyran-2-yloxy)chalcone (**C2**, 703 mg, 2.0 mmol), 3-aminocrotononitrile (328 mg, 4.0 mmol), NaOH (80 mg, 2.0 mmol), and 160 mL of EtOH were combined. The solution was refluxed for 29 h. The condenser was removed, and the solution was stirred at room temperature under an oxygen atmosphere for 12 h. The precipitate was filtered and allowed to air dry. This procedure generated 366 mg of **C10** as a white solid. 44% yield. TLC: R_f=0.33 (20% ethyl acetate in hexane); Melting point: 148-150 °C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.02, 7.17 (AA'XX' peak, $J_{AA'}$=$J_{XX'}$=2.2, J_{AX}=8.6, $J_{AX'}$=0.1 Hz, 4H), 7.60, 6.81 (AA'XX' peak, $J_{AA'}$=$J_{XX'}$=2.3, J_{AX}=8.6, $J_{AX'}$=0.2 Hz, 4H), 7.59 (s, 1H), 5.51 (m, 1H), 3.90 (m, 1H), 3.63 (m, 2H), 3.05 (s, 6H), 2.85 (s, 3H), 2.09 (m, 2H), 1.90 (m, 2H), 1.67 (m, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 207.2, 162.7, 158.9, 158.5, 153.8, 151.5, 131.6, 129.6, 128.9, 123.8, 118.4, 116.8, 116.5, 112.1, 104.2. 96.3, 62.2, 40.3, 30.7, 30.5, 30.4, 30.2, 30.0, 29.7, 25.3, 24.5, 18.8; IR (ATR): 3195, 2924, 2221, 1679, 1582, 1529, 1435, 1370, 1310, 1241, 1199, 1180, 1142, 1054, 1033 cm^{-1}; ESI-MS: expected, 413.2; observed, m/z 414.2 [M+H$^+$].

3-cyano-4-(4-dimethylaminophenyl)-6-(4-hydroxyphenyl)-2-methylpyridine (3f). In a 4 mL vial, 3-cyano-4-(4-dimethylaminophenyl)-6-(4-(tetrahydro-2*H*-pyran-2-yloxy)phenyl)-2-methyl-pyridine (**C10**, 150 mg, 0.36 mmol) was dissolved in 1.0 mL of 20% trifluoroacetic acid in CH_2Cl_2 were combined. The vial was closed with a Teflon-lined cap, shaken, and allowed to stand for 5 min. The solution was concentrated to a dark orange oil under a stream of N_2. Trituration with 0.5 mL of diethyl ether produced 100 mg of **3f** as a dark red solid. 83% yield. TLC: R_f=0.22 (40% ethyl acetate in hexane); Melting point: 144-145 °C; ^{1}H-NMR (300 MHz, DMSO): δ 8.07, 6.87 (AA'XX' peak, $J_{AA'}$=$J_{XX'}$=2.4, J_{AX}=13.6, $J_{AX'}$=5.1 Hz, 4H), 7.76 (s, 1H), 7.61, 6.84 (AA'XX' peak, $J_{AA'}$=$J_{XX'}$=2.3, J_{AX}=8.9, $J_{AX'}$=0.1 Hz, 4H), 3.00 (s, 6H), 2.74 (s, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 162.5, 160.4, 158.3, 153.7, 151.9, 130.2, 129.8, 128.7, 123.7, 118.8, 116.3, 116.2, 112.6, 103.4, 31.3, 24.7; IR (ATR): 3195, 2973, 2230, 1678, 1596, 1582, 1528, 1436, 1371, 1241, 1197, 1180, 1142, 1033 cm^{-1}; ESI-MS: expected, 329.2; observed, m/z 330.0 [M+H$^+$].

3-cyano-4-(4-methoxyphenyl)-6-(4-hydroxyphenyl)-2-methylpyridine (3e). 4'-hydroxy-4-methoxychalcone (**C3**, 508 mg, 2.0 mmol), 3-aminocrotononitrile (328 mg, 4.0 mmol), NaOH (80 mg, 2.0 mmol), and 40 mL of EtOH were combined in a 70 mL Teflon/PEEK Milestone microwave reaction vessel. The reaction vessel was tightly closed and heated (with stirring)

using a Milestone microwave reactor from room temperature to 150 °C over 15 min, held at 150 °C for 20 min, and allowed to cool to room temperature. The solution was poured into a 100 mL round bottom flask. A 4.0 mL aliquot of 1N aq. HCl was added. The solution was stirred at room temperature under an oxygen atmosphere for 12 h. The precipitate was filtered and allowed to air dry. This procedure generated 110 mg of **3e** as a white solid. 9% yield. TLC: R_f=0.14 (20% ethyl acetate in hexane); Melting point: 220-221 °C; ^{1}H-NMR (300 MHz, Acetone-d6) δ 8.18, 6.99 (AA'XX' peak, $J_{AA'}$=$J_{XX'}$=2.5, J_{AX}=8.5, $J_{AX'}$=0.3 Hz, 4H), 7.83 (s, 1H), 7.72, 7.14 (AA'XX' peak, $J_{AA'}$=$J_{XX'}$=2.6, J_{AX}=8.5, $J_{AX'}$=0.3 Hz, 4H), 3.85 (s, 3H), 2.75 (s, 3H); ^{13}C-NMR (75 MHz, DMSO-d6) δ 162.4, 161.2, 160.6, 158.6, 153.2, 130.8, 129.8, 129.1, 128.5, 118.4, 116.8, 116.3, 114.9, 104.1, 56.1, 24.7; IR (ATR): 3195, 2973, 2221, 1610, 1587, 1533, 1517, 1462, 1382, 1303, 1284, 1252, 1176, 1119, 1048, 1033 cm^{-1}; ESI-MS: expected, 316.1; observed, m/z 316.9 [M+H$^+$].

1,3-dimethyl-5-(4-fluorophenyl)-7-(4-hydroxyphenyl)-pyrido[2,3-d]pyrimidine-2,4 (1H, 3H)-dione (6c). 4'-fluoro-4-hydroxychalcone (**C4**, 480 mg, 2.0 mmol), 6-amino-2,3-dimethyluracil (**4**, 310 mg, 2.0 mmol), KOH (112 mg, 2.0 mmol), and 4.0 mL of EtOH were combined in a 20 mL vial. The vial was purged with oxygen, sealed with a Teflon-lined cap, and heated at 80 °C in a conventional drying oven for 16 h. The mixture was allowed to cool to room temperature, after which 2.0 g of ice was added. After stirring for 15 min, the mixture was filtered and washed with 1.0 mL of water and 1.0 mL of EtOH. This procedure generated 113 mg of **6c** as a yellow solid. 15% yield. TLC: R_f=0.28 (40% ethyl acetate in hexane); ^{1}H-NMR (300 MHz, DMSO-d6) δ 7.97 (d, 8.9 Hz, 2H), 7.39 (m, 2H), 7.32 (s, 1H), 7.21 (m, 2H), 6.60 (d, J=8.9 Hz, 2H), 3.66 (s, 3H), 3.15 (s, 3H); ^{13}C-NMR (75 MHz, DMSO-d6) δ 169.4, 162.5 (d, J=244.4 Hz), 160.5, 159.8, 152.7, 152.1, 136.8, 131.0 (d, J=8.2 Hz), 130.1, 122.0, 118.4, 116.3, 114.8 (d, J=21.4 Hz), 104.0, 30.3, 28.5; ESI-MS: expected, 377.12; observed, m/z 378.1 [M+H$^+$].

1,3-dimethyl-5-(4-fluorophenyl)-7-(4-methoxyphenyl)-pyrido[2,3-d]pyrimidine-2,4(1H, 3H)-dione (6t). Synthesized according to the above method for **6c**: 460 mg. 59% yield. TLC: R_f=0.14 (20% ethyl acetate in hexane); Melting point: 240-243 °C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.0, 7.01 (AA'XX' peak, $J_{AA'}$=$J_{XX'}$=2.5, J_{AX}=8.7, $J_{AX'}$=0.2 Hz, 4H), 7.39 (s, 1H), 7.32 (m, 2H), 7.15 (m, 2H), 3.89 (s, 3H), 3.87 (s, 3H), 3.39 (s, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 163.2, 162.7, (d, J=246 Hz), 162.0, 160.6, 159.0, 153.9, 151.7 (d, J=22.4 Hz), 135.5 (d, J=2.9 Hz), 129.7 (d, J=8.3 Hz), 129.1, 117.6, 115.0, 114.8, 114.4, 105.8, 55.5, 30.1, 28.4; IR (ATR): 2989, 2896, 1702, 1660, 1600, 1457, 1439, 1422, 1367, 1287, 1263, 1221, 1165, 1086 cm^{-1}; ESI-MS: expected, 377.12; observed, m/z 378.1 [M+H$^+$].

Derivatization of planar cellulose supports.

Representative planar cellulose membrane amination protocol. Dots were marked on a 14 cm × 18 cm sheet of Whatman 1Chr paper at distances 1.4 cm apart using a #2 lead pencil. The sheet was immersed in 100 mL of 10% TFA in CH_2Cl_2 for 10 min in a covered 2.6 L Pyrex dish. This acid wash serves as a cellulose preactivation step and is believed to increase the surface area of the cellulose available for functionalization.[6] The sheet was washed by adding 60 mL of CH_2Cl_2, allowing it soak for 5 min, then decanting the CH_2Cl_2. This process was repeated, after which the membrane was dried under a stream of air for 10 min. The sheet was next immersed in 50 mL of 2M tosyl chloride in pyridine for 2 h. The paper was washed by immersion in three consecutive baths of EtOH (100 mL, 5 min each) and dried under a stream of N_2.

To install the flexible amine space, the tosylated cellulose paper was immersed in 60 mL of neat 4,7,10-trioxa-1,13-tridecanediamine[7] and placed on the rotating platform of the Milestone microwave reactor. The dish was covered with a second Pyrex dish. The sheet was irradiated for 15 min at 400 W. The amine solution was carefully decanted from the paper. The paper was washed by adding then decanting 70 mL portions of DMF, EtOH, 1.0N NaOH, H_2O, EtOH (2x), and CH_2Cl_2 (5 min in each bath). The amino paper was then dried under a stream of N_2.

Representative Fmoc quantitation protocol on cellulose supports. A spot (6 mm diameter) was punched from amino cellulose support using a desktop hole punch and immersed in 200 μL of 0.60 M Fmoc-OSu in DMF for 2 h. The spot was washed with 10 mL of EtOH, 10 mL of acetone, and 10 mL of CH_2Cl_2. After drying under a stream of N_2, 960 μL of DMF was added followed by 40 μL of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). The mixture was swirled for 30 sec and then allowed to stand for 15 min. The mixture was swirled again for 30 sec, then 250 μL of this solution was removed and diluted with 1.0 mL of DMF. The solution was swirled again for 30 sec. The absorbance was read at 296 nm (ε_{296}= 9500 $M^{-1}cm^{-1}$) in a quartz cuvette. The value was multiplied by 5 to account for the dilution.[8] Loadings of 1.0-10 μmol/cm^2 were obtained using this method. Longer tosylation reaction times gave higher levels of functionalization (*e.g.*, 2 h = 4.0 μmol/cm^2, 12 h = 10 μmol/cm^2).

Representative Wang-linker installation procedure on amino cellulose support. 4-formylphenoxyacetic acid (5.40 g, 30.0 mmol), diisopropylcarbodiimide (DIC, 4.7 mL, 30.0 mmol), *N*-hydroxysuccinimide (3.45 g, 30.0 mmol), NEt_3 (4.2 mL, 30.0 mmol), and DMF (50 mL) were combined in a 2.6 L Pyrex dish. The dish was covered and swirled for 30 min at room temperature. A 14 cm × 18 cm sheet of amino cellulose support was added. The dish was covered again and the mixture was swirled at room temperature for 2 h. The coupling solution was decanted.[9] The paper was washed by adding then decanting 70 mL portions of DMF (2x), EtOH (2x), and CH_2Cl_2 (5 min in each bath). The aldehyde-derived paper was then dried under a stream of N_2.

A 100 mL aliquot of 1.0 M $NaBH_4$ in 1.0 M aq. NaOH was added to the aldehyde-derived support. The mixture was swirled for 20 min, after which the $NaBH_4$ solution was decanted. The paper was then washed by adding then decanting 70 mL portions of H_2O (2x), EtOH (2x), and CH_2Cl_2 (5 min in each bath). The benzyl alcohol paper was dried under a stream of N_2. To approximate the linker loading, the amount of residual amine was measured by Fmoc quantitation as described above, except a 2-3-fold dilution was used instead. Residual amine loadings are *ca*. 600-800 nmol/cm^2.

Representative Wang-linker activation protocol. Immediately prior to use, a 14 cm × 18 cm sheet of benzyl alcohol paper was activated by submerging it in 50 mL of a 4.0 M solution of tosyl chloride in dry DMF and swirled for 30 min on an orbital shaker. The paper was washed by immersion and swirling in dry CH_2Cl_2 (70 mL, 3 x 2 min). The benzyl chloride-derived paper was then dried by a stream of N_2 for 10 min prior to the next step.

Synthesis of chalcone macroarray 1.

Coupling of hydroxyacetophenones to activated planar support. A 2.0 M coupling solution of the various hydroxyacetophenones solutions were prepared by adding an equal volume of a 4.0 M solution of KO*t*Bu in anhydrous DMF to a 4.0 M solution of substituted hydroxyacetophenone in anhydrous DMF. Twelve 3.0 μL aliquots of each solution was applied to activated Wang-linker derived cellulose as twelve spots at distances 1.4 cm apart along three rows (**A**-**C**):

Row A: 4'-hydroxyacetophenone
Row B: 3'-hydroxyacetophenone
Row C: acetovanillone

The spotted planar support was subjected to microwave irradiation in a Pyrex dish at 500 W for 10 min in the Milestone microwave reactor. The support was next washed in a Pyrex dish by adding then decanting 70 mL portions of 1N NaOH, H_2O (2x), EtOH (2x), and CH_2Cl_2 (5 min in each bath). The acetophenone-derived support was dried under a stream of N_2 (for 10 min) prior to the next step.

Claisen-Schmidt condensation on planar support. The following benzaldehyde solutions were prepared and three aliquots were spotted onto acetophenones-derived support at distances 1.4 cm apart down the corresponding columns (**1**-**12**):

Column 1: 6.0 μL of 1.0 M benzaldehyde in 1.5 N NaOH in 50% aq. EtOH.
Column 2: 6.0 μL of 0.5 M 2-fluorobenzaldehyde in 1.5 N NaOH in 50% aq. EtOH.
Column 3: 6.0 μL of 1.0 M 3-fluorobenzaldehyde in 1.5 N NaOH in 50% aq. EtOH.
Column 4: 6.0 μL of 1.0 M 4-fluorobenzaldehyde in 1.5 N NaOH in 50% aq. EtOH.
Column 5: 6.0 μL of 0.5 M 3-bromobenzaldehyde in 1.5 N NaOH in 50% aq. EtOH.
Column 6: 6.0 μL of 0.5 M 4-bromobenzaldehyde in 1.5 N NaOH in 50% aq. EtOH.
Column 7: 6.0 μL of 0.5 M 4-chlorobenzaldehyde in 1.5 N NaOH in 50% aq. EtOH.
Column 8: 6.0 μL of 1.0 M 3,4-difluorobenzaldehyde in 1.5 N NaOH in 50% aq. EtOH.
Column 9: 6.0 μL of 0.5 M *m*-anisaldehyde in 1.5 N NaOH in 50% aq. EtOH.
Column 10: 6.0 μL of 1.0 M *p*-anisaldehyde in 1.5 N NaOH in 50% aq. EtOH.
Column 11: 6.0 μL of 1.0 M 3-chlorobenzaldehyde in 1.5 N NaOH in 50% aq. EtOH.
Column 12: 6.0 μL of 1.0 M 4-dimethylaminobenzaldehyde in warm ethylene glycol.

To column 12, 8.0 μL of 5.0 M KO*t*Bu in ethylene glycol was added. (Since 4-dimethylaminobenzaldehyde was insoluble in high concentrations of base, the solution of aldehyde and the solution of the base were added separately). The spotted support was placed in

a Pyrex dish in the Milestone microwave reactor and irradiated at 400 W for 10 min. Columns 1-12 were then respotted with the benzaldehyde solutions and irradiated a second time in the Milestone microwave reactor at 400 W for 10 min. The support was next washed in a Pyrex dish by adding then decanting 70 mL portions of 1% aq. AcOH, DMSO, EtOH (2x), and CH_2Cl_2 (5 min in each bath). The chalcone-derived support was dried under a stream of N_2.

Synthesis of fluorescent dye macroarrays 2 and 5.

Cyanopyridine macroarray (2): KO*t*Bu (1.234 g, 11.0 mmol) was suspended in 5.0 mL of acetonitrile in a 20 mL vial. The mixture was capped with a Teflon-lined cap and sonicated in an ultrasound bath (Branson model # 1510R-MT) for 50 min. After sonication, 6.0 μL aliquots of the mixture were applied to each spot on chalcone array **1**. The membrane was allowed to stand at room temperature for 10 min. The spotting and standing steps were repeated three more times. The membrane was washed in a Pyrex dish by adding then decanting 70 mL portions of DMSO, 1% aq. acetic acid, EtOH (2x), and CH_2Cl_2 (5 min in each bath). The cyanopyridine array (**2**) was then dried under a stream of N_2.

Deazalumazine macroarray (5): 6-amino-2,3-dimethyl uracil (**4**, 77.6 mg, 0.5 mmol) was dissolved in 1.5 mL of DMSO and 0.5 mL of 1.0 N aq. NaOH. A 6.0 μL aliquot of this solution was applied to each spot on chalcone array **1**. The support was placed in a Pyrex dish in the Milestone microwave reactor and irradiated at 400 W for 10 min. The spotting and microwave irradiation steps were repeated three more times. The membrane was washed in a Pyrex dish by adding then decanting 70 mL portions of DMSO, 1% aq. acetic acid, EtOH (2x), and CH_2Cl_2 (5 min in each bath). The deazalumazine array (**5**) was then dried under a stream of N_2.

Vapor-phase cleavage of dye compounds from macroarrays. This cleavage method is a modified version of that reported by Scharn *et al.*[10] Compound spots were punched out from the macroarray with a desktop hole-punch and placed in individual vials. TFA (10 mL) was added to the bottom of a glass vacuum dessicator (26 cm diameter, 20 cm tall). A Petri dish (14.5 cm diameter) was placed on a ceramic platform above the TFA, and the vials containing the macroarray members were placed on this dish. The dessicator was evacuated to 60 mbar for 10 min and then sealed for 50 min. The seal was broken, and the vials then were removed from of the dessicator. The vials were allowed to stand in a fume hood at room temperature for 30 min, after which 1.0 mL of EtOH was added to each vial. After swirling for 15 min, 0.5 mL of each solution was removed for LC-MS analyses and placed in separate glass vials. The remaining 0.5 mL portions were used for spectral characterization of the macroarray members.

LC-MS analysis of dye compounds. The EtOH solutions were concentrated under reduced pressure using a SpeedVac centrifuge system. The resulting residues were dissolved in 150 μL of 50% aqueous acetonitrile, filtered through a cotton plug, and analyzed by LC-MS. HPLC: conditions as described above, solvent gradient 20-95% B in 8 min followed by 4 min at 95% B. MS: (ESI) positive ion mode was used for all samples. Relative conversions (in comparison to acetophenone and chalcone starting materials) and compound purities were determined by integration of peaks in the HPLC trace at 254 nm using the Shimadzu LCMSolution software package.

Spectral characterization methods.

Screening of macroarrays and fluorophore solutions using a handheld UV-lamp. Dried macroarrays or solutions (in 4.0 mL Pyrex vials) were placed under a handheld UV-lamp (C. Entela Mineralight® Lamp; model UVGL-58). Irradiation of the macroarrays at 254 nm yielded the highest contrast between spots. Solutions in Pyrex vials were irradiated at 366 nm, as Pyrex blocks shortwave UV (254 nm). A digital camera (Canon PowerShot SD500) was used to photograph the arrays and solutions, using the "daylight white balance" setting. On occasion, recording the irradiated samples in the "filming mode" and copying a specific frame using Microsoft Windows Media Player® (V 10.0) provided the most accurate photographs.

Standard preparation. A stock solution of the fluorescence standard coumarin 120 (1 mg in 4.0 mL of EtOH) was diluted with EtOH until the absorbance at 340 nm was between 0.05 and 0.01.[11] The fluorescence spectra were recorded using an excitation wavelength of 340 nm. The fluorescence emission was integrated with Microsoft Excel. This was repeated three times at different optical densities.

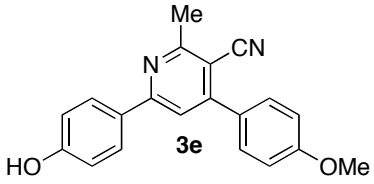
Evaluation of macroarray compounds. Each cleaved spot sample was diluted with EtOH until the absorbance at 340 nm was between 0.05 and 0.01. The fluorescence spectra were recorded with an excitation wavelength of 340 nm. The peak was integrated using Microsoft Excel. This process was repeated three times at different optical densities. The integrated intensity was plotted against the absorbance of each sample. The slopes of the resulting lines were calculated using Microsoft Excel. The quantum yield was then determined by the following equation:[1]

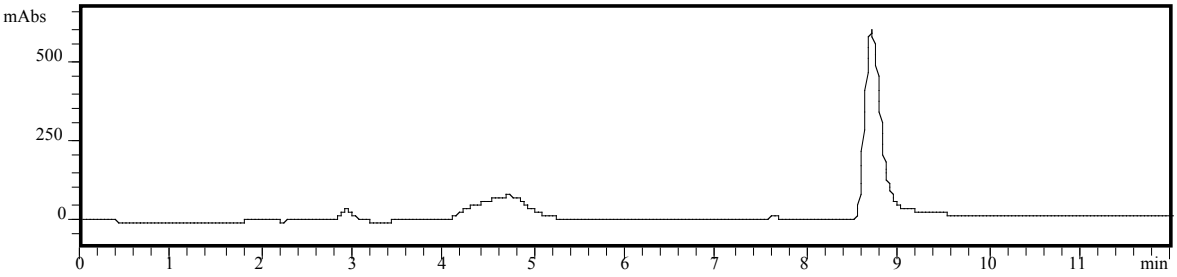
$$\phi_{sample} = \phi_{std} \frac{(Slope_{sample})(\eta^2_{sample})}{(Slope_{std})(\eta^2_{std})}$$

Evaluation of solution-phase standards. Stock solutions of fluorophores prepared by solution-phase synthesis were made at the approximate concentration of 1 mg in 4.0 mL of solvent. Aliquots of the stock solutions were diluted until the absorbance at 340 nm was between 0.05 and 0.01. The fluorescence spectra were recorded with an excitation wavelength of 340 nm. Quantum yields were calculated as described above.[1]

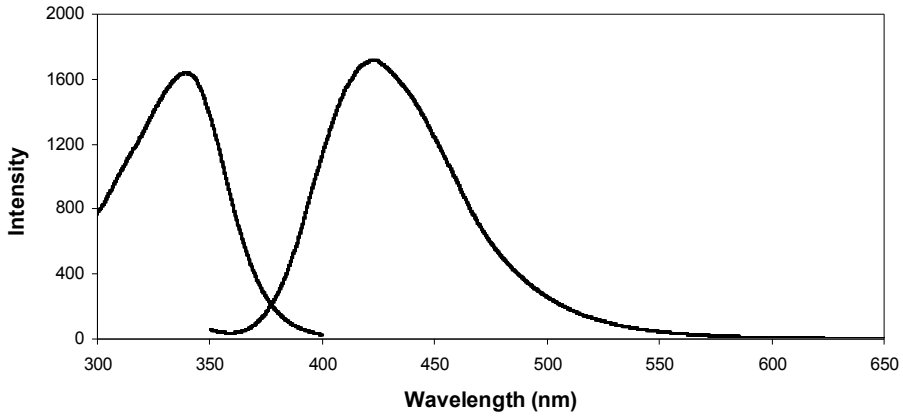
<u>Representative spectral characterization data for 3e, 3f, and 6c.</u>

Characterization of cyanopyridine 3e.

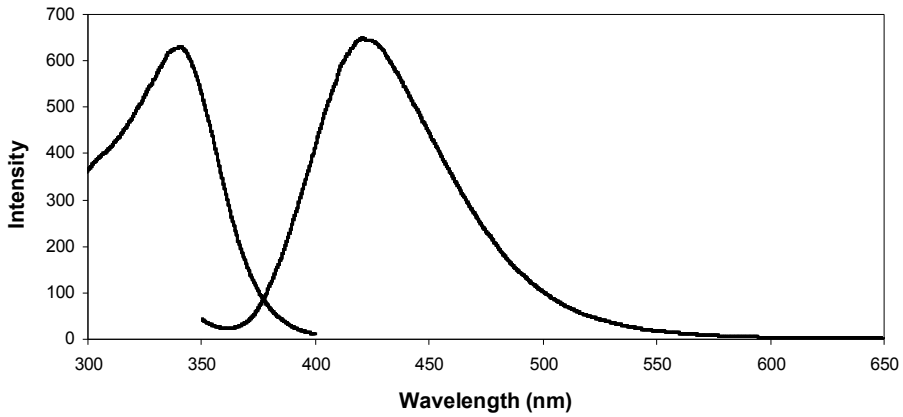




HPLC trace of crude **3e** obtained from macroarray (254 nm).

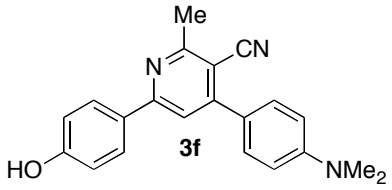


Excitation and emission spectra of crude **3e** obtained from macroarray (in EtOH).

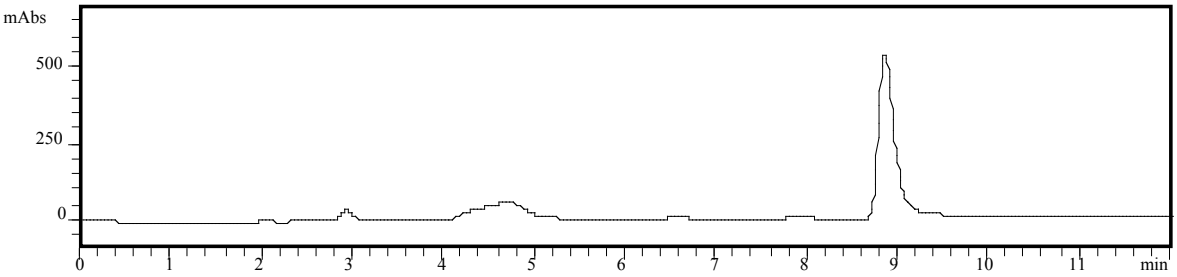


Excitation and emission spectra of purified **3e** obtained from solution-phase synthesis (in EtOH).

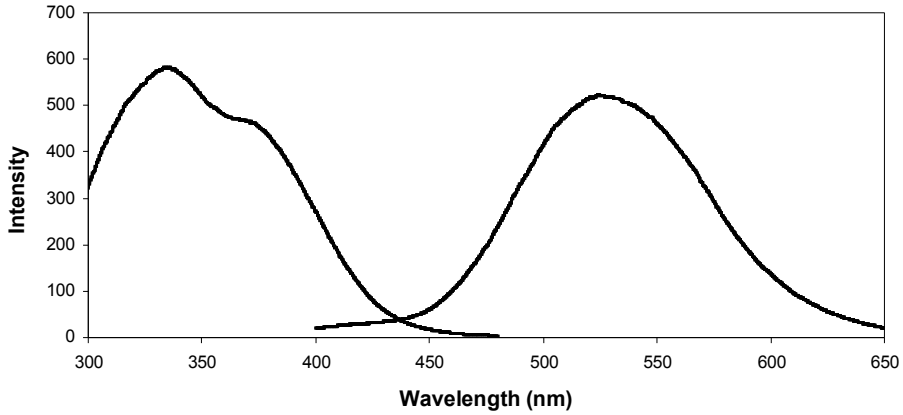
Characterization of cyanopyridine 3f.



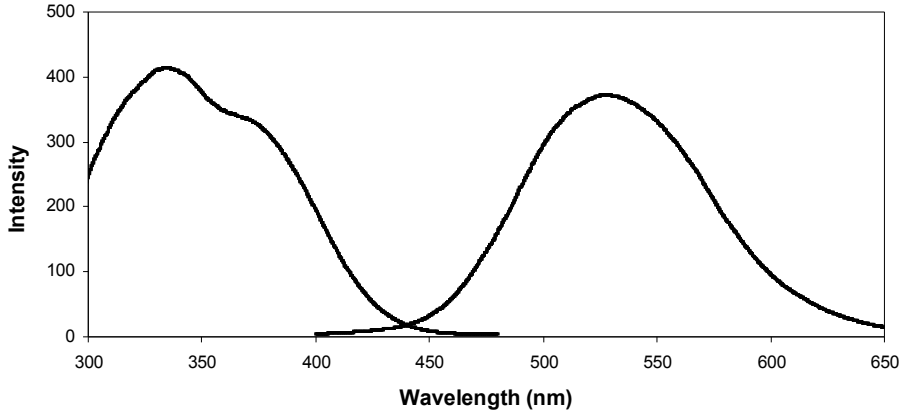
3f



HPLC trace of crude **3f** obtained from macroarray (254 nm).

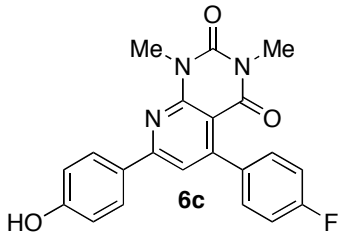


Excitation and emission spectra of crude **3f** obtained from macroarray (in EtOH).

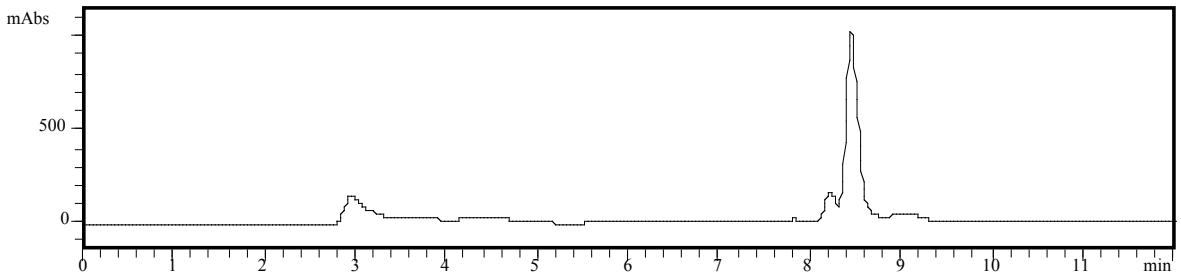


Excitation and emission spectra of purified **3f** obtained from solution-phase synthesis (in EtOH).

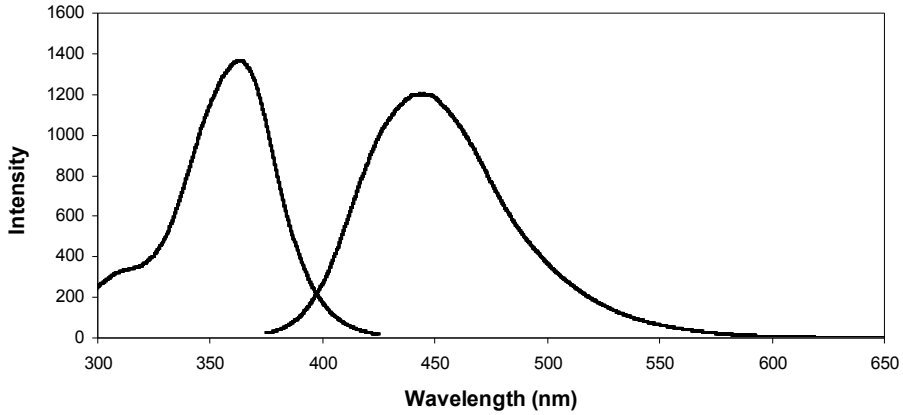
Characterization of deazalumazine 6c.



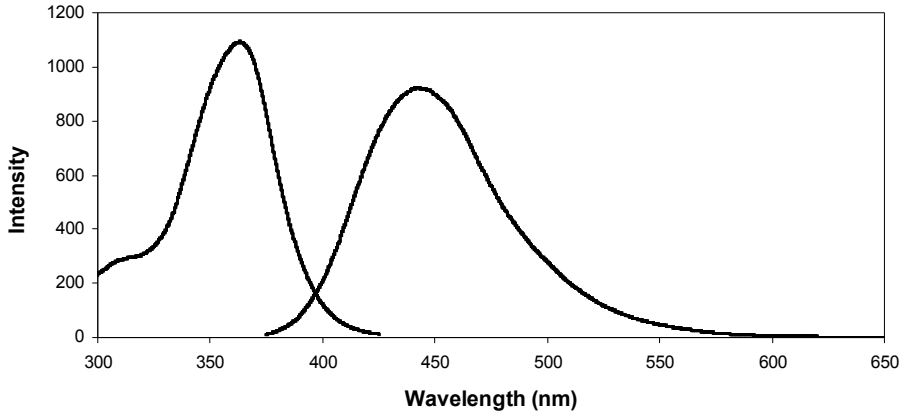
6c



HPLC trace of crude **6c** obtained from macroarray (254 nm).

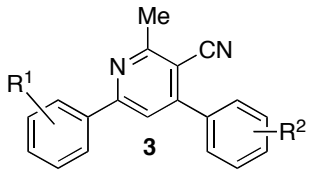


Excitation and emission spectra of crude **6c** obtained from macroarray (in EtOH).



Excitation and emission spectra of purified **6c** obtained from solution-phase synthesis (in EtOH).

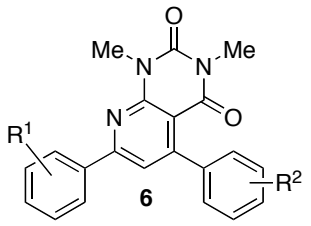
LC-MS analysis of cyanopyridines (3) cleaved from macroarray 2.



entry[a]	R^1	R^2	retention time (min)	calc. mass	obs. mass	purity (%)[b]
3a	4-OH	H	8.713	286.1	286.9 $[M+H]^+$	81
3b	4-OH	2-F	8.629	304.1	304.9 $[M+H]^+$	81
3c	4-OH	4-F	8.839	304.1	304.9 $[M+H]^+$	83
3d	4-OH	4-Br	9.553	364.0	365.0 $[M+H]^+$	82
3e	4-OH	4-OMe	8.869	316.1	316.9 $[M+H]^+$	70
3f	4-OH	4-NMe_2	8.846	329.2	329.9 $[M+H]^+$	74
	4-OH	3-F	8.859	304.1	304.9 $[M+H]^+$	78
3g	3-OMe, 4-OH,	H	8.838	316.1	316.9 $[M+H]^+$	74
3h	3-OMe, 4-OH,	3-OMe	8.932	346.1	346.9 $[M+H]^+$	89
3i	3-OMe, 4-OH,	4-F	8.952	334.1	335.0 $[M+H]^+$	71
3j	3-OMe, 4-OH,	4-Br	9.698	394.0	394.9 $[M+H]^+$	70
3k	3-OMe, 4-OH,	4-OMe	8.852	346.1	346.9 $[M+H]^+$	78
3l	3-OMe, 4-OH,	4-NMe_2	9.020	359.2	359.9 $[M+H]^+$	87
	3-OMe, 4-OH,	2-F	8.792	334.1	334.9 $[M+H]^+$	91
	3-OMe, 4-OH,	3-F	9.030	334.1	334.9 $[M+H]^+$	89
3m	3-OH	4-OMe	8.739	316.1	317.0 $[M+H]^+$	84
	3-OH	3-Br	9.496	364.0	364.8 $[M+H]^+$	77
	3-OH	4-Br	9.617	364.0	364.8 $[M+H]^+$	86
	3-OH	3-Cl	9.476	320.1	320.9 $[M+H]^+$	85
	3-OH	3-F, 4-F	9.097	322.1	322.9 $[M+H]^+$	83

[a] Library members without compound numbers represent compounds that were omitted from the text for the sake of brevity. [b] Determined by HPLC integration at 254 nm.

LC-MS analysis of deazalumazines (6) cleaved from macroarray 5.

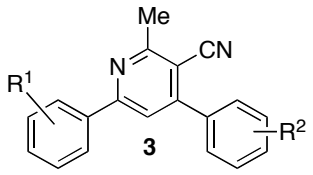


6

entry[a]	R^1	R^2	retention time (min)	calc. mass	obs. mass	purity (%)[b]
6a	4-OH	H	8.269	359.1	360.1 $[M+H]^+$	77
6b	4-OH	2-F	8.232	377.1	377.9 $[M+H]^+$	79
6c	4-OH	4-F	8.433	377.1	377.9 $[M+H]^+$	78
6d	4-OH	4-Br	9.129	437.0	438.0 $[M+H]^+$	78
6e	4-OH	4-OMe	8.210	389.1	390.0 $[M+H]^+$	82
	4-OH	4-Cl	9.014	393.1	393.8 $[M+H]^+$	79
	4-OH	4-Br	9.166	437.0	438.0 $[M+H]^+$	87
	4-OH	3-F	8.433	377.1	378.1 $[M+H]^+$	87
	4-OH	3-Cl	8.955	393.1	393.9 $[M+H]^+$	86
6g	3-OMe, 4-OH	H	8.440	389.1	390.1 $[M+H]^+$	82
6h	3-OMe, 4-OH	3-OMe	8.455	419.2	419.9 $[M+H]^+$	78
6i	3-OMe, 4-OH	4-F	8.573	407.1	408.0 $[M+H]^+$	76
6j	3-OMe, 4-OH	4-Br	9.371	467.1	468.0 $[M+H]^+$	76
6k	3-OMe, 4-OH	4-OMe	8.358	419.2	420.1 $[M+H]^+$	77
6r	3-OH	4-F	8.594	377.1	377.9 $[M+H]^+$	87
6s	3-OH	4-Cl	9.182	393.1	394.1 $[M+H]^+$	84
	3-OH	3-Br	9.250	437.0	437.9 $[M+H]^+$	95
	3-OH	4-Br	9.319	437.0	437.9 $[M+H]^+$	86
	3-OH	H	8.436	359.1	360.1 $[M+H]^+$	79

[a] Library members without compound numbers represent compounds that were omitted from the text for the sake of brevity. [b] Determined by HPLC integration at 254 nm.

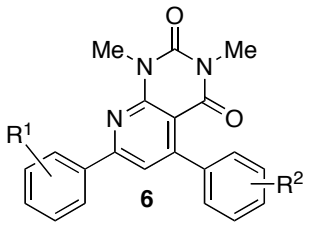
Spectral data for cyanopyridines 3.



entry[a]	R^1	R^2	λ_{ex}	λ_{em}	ϕ_f[b]	entry[a]	R^1	R^2	λ_{ex}	λ_{em}	ϕ_f[b]
3a	4-OH	H	341	433	0.07	**3d**	4-OH	4-Br	342	440	0.04
3b	4-OH	2-F	342	430	0.06		4-OH	4-Cl	343	443	0.04
	4-OH	3-F	334	445	0.04		4-OH	3-Cl	343	446	0.03
3c	4-OH	4-F	341	430	0.07		4-OH	3-OMe	341	433	0.05
	4-OH	3F, 4-F	343	441	0.03	**3e**	4-OH	4-OMe	339	423	0.12
	4-OH	3-Br	344	445	0.03	**3f**	4-OH	4-NMe_2	366	524	0.09
3g	3-OMe, 4-OH	H	351	469	0.03	**3j**	3-OMe, 4-OH	4-Br	352	475	0.02
	3-OMe, 4-OH	2-F	351	465	0.02		3-OMe, 4-OH	4-Cl	351	474	0.02
	3-OMe, 4-OH	3-F	356	476	0.01		3-OMe, 4-OH	3-Cl	355	485	0.01
3i	3-OMe, 4-OH	4-F	351	466	0.04	**3h**	3-OMe, 4-OH	3-OMe	351	473	0.03
	3-OMe, 4-OH	3F, 4-F	355	471	0.02	**3k**	3-OMe, 4-OH	4-OMe	351	461	0.06
	3-OMe, 4-OH	3-Br	353	477	0.02	**3l**	3-OMe, 4-OH	4-NMe_2	348	533	0.09
	3-OH	H	336	447	<0.01		3-OH	4-Br	343	451	<0.01
	3-OH	2-F	325	445	<0.01		3-OH	4-Cl	341	447	<0.01
	3-OH	3-F	338	443	<0.01		3-OH	3-OMe	338	442	<0.01
	3-OH	4-F	335	444	<0.01	**3m**	3-OH	4-OMe	326	420	<0.01
	3-OH	3F, 4-F	341	448	<0.01		3-OH	4-NMe_2	377	542	0.05
	3-OH	3-Br	347	443	<0.01	**3n**[c]	4-OMe	2-F	336	419	0.71
3o[c]	4-OMe	4-OMe	335	409	0.77	**3q**[c]	3,4-OMe	4-OMe	344	448	0.74
3p[c]	4-OMe	3-OMe	306	417	0.38						

[a] Library members without compound numbers represent compounds that were omitted from the text for the sake of brevity. [b] Data recorded from crude samples. Quantum yields measured in EtOH. External standard: coumarin 120 (ϕ_f=0.88, λ_{ex}=354 nm, λ_{em}=435 nm in EtOH). Error = ±15%. [c] Synthesized in solution and purified.

Spectral data for deazalumazines 6.

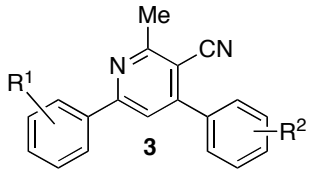


6

entry[a]	R^1	R^2	λ_{ex}	λ_{em}	ϕ_f[b]	entry[a]	R^1	R^2	λ_{ex}	λ_{em}	ϕ_f[b]
6a	4-OH	H	362	446	0.15	**6d**	4-OH	4-Br	364	450	0.09
6b	4-OH	2-F	363	443	0.18		4-OH	4-Cl	364	449	0.10
	4-OH	3-F	364	450	0.15		4-OH	3-Cl	363	451	0.13
6c[c]	4-OH	4-F	364	445	0.19		4-OH	3-OMe	363	444	0.04
	4-OH	3F, 4-F	364	450	0.12	**6e**	4-OH	4-OMe	365	438	0.03
	4-OH	3-Br	364	452	0.07		3-OMe, 4-OH	3-Br	370	489	0.03
6g	3-OMe, 4-OH	H	368	480	0.06	**6j**	3-OMe, 4-OH	4-Br	372	495	0.05
	3-OMe, 4-OH	2-F	371	485	0.06		3-OMe, 4-OH	4-Cl	371	492	0.05
	3-OMe, 4-OH	3-F	370	493	0.05		3-OMe, 4-OH	3-Cl	372	496	0.04
6i	3-OMe, 4-OH	4-F	370	483	0.07	**6h**	3-OMe, 4-OH	3-OMe	370	482	0.05
	3-OMe, 4-OH	3F, 4-F	371	492	0.05	**6k**	3-OMe, 4-OH	4-OMe	372	474	0.06
	3-OH	H	363	441	<0.01		3-OH	4-Br	355	442	<0.01
	3-OH	2-F	365	439	<0.01	**6s**	3-OH	4-Cl	356	440	<0.01
	3-OH	3-F	354	455	<0.01		3-OH	3-OMe	347	428	<0.01
6r	3-OH	4-F	367	441	0.05		3-OH	4-OMe	351	456	0.01
	3-OH	3F, 4-F	361	446	<0.01		3-OH	3-Br	352	440	<0.01
6t[c]	4-OMe	4-F	360	424	0.19[b]						

[a] Library members without compound numbers represent compounds that were omitted from the text for the sake of brevity. [b] Data recorded from crude samples. Quantum yields measured in EtOH. External standard: coumarin 120 (f_f=0.88, l_{ex}=354 nm, l_{em}=435 nm in EtOH). Error = ±15%. [c] Synthesized in solution and purified.

Dependence of the spectral properties of cyanopyridines 3e, 3f, 3n, and 3o on pH.

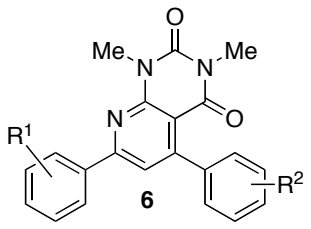


3

compound[a]	R^1	R^2	buffer (in EtOH)	pH	λ_{ex}	λ_{em}	ϕ_f[b]
3e	4-OH	4-OMe	0.1 M TFA	1.6	342	422	0.14
3e	4-OH	4-OMe	0.1 M AcOH	4.8	339	423	0.14
3e	4-OH	4-OMe	0.1 M NH_4OAc	7.6	340	423	0.12
3e	4-OH	4-OMe	0.1 M NH_4OAc/KOH (1:1)	10.0	340	421	0.09
3f	4-OH	4-NMe_2	0.1 M TFA	1.6	336	526	0.06
3f	4-OH	4-NMe_2	0.1 M AcOH	4.8	336	530	0.11
3f	4-OH	4-NMe_2	0.1 M NH_4OAc	7.6	335	529	0.09
3f	4-OH	4-NMe_2	0.1 M NH_4OAc/KOH (1:1)	10.0	336	528	0.09
3n	4-OMe	2-F	0.1 M TFA	1.6	337	419	0.64
3n	4-OMe	2-F	0.1 M AcOH	4.8	336	419	0.65
3n	4-OMe	2-F	0.1 M NH_4OAc	7.6	336	418	0.71
3n	4-OMe	2-F	0.1 M NH_4OAc/KOH (1:1)	10.0	336	420	0.68
3o	4-OMe	4-OMe	0.1 M TFA	1.6	335	408	0.78
3o	4-OMe	4-OMe	0.1 M AcOH	4.8	337	407	0.76
3o	4-OMe	4-OMe	0.1 M NH_4OAc	7.6	337	408	0.74
3o	4-OMe	4-OMe	0.1 M NH_4OAc/KOH (1:1)	10.0	336	408	0.76

[a] Data obtained from compounds synthesized in solution and purified. [b] External standard: coumarin 120 (ϕ_f=0.88, λ_{ex}=354 nm, λ_{em}=435 nm in EtOH). Error ±10%.

Dependence of the spectral properties of deazalumazines 6c and 6t on pH.

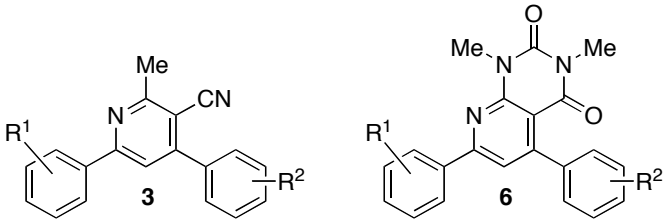


6

compound[a]	R^1	R^2	buffer (in EtOH)	pH	λ_{ex}	λ_{em}	ϕ_f[b]
6c	4-OH	4-F	0.1 M TFA	1.6	362	444	0.25
6c	4-OH	4-F	0.1 M AcOH	4.8	363	444	0.25
6c	4-OH	4-F	0.1 M NH_4OAc	7.6	362	443	0.20
6c	4-OH	4-F	0.1 M NH_4OAc/KOH (1:1)	10.0	362	442	0.16
6t	4-OMe	4-F	0.1 M TFA	1.6	359	424	0.18
6t	4-OMe	4-F	0.1 M AcOH	4.8	360	423	0.18
6t	4-OMe	4-F	0.1 M NH_4OAc	7.6	360	425	0.20
6t	4-OMe	4-F	0.1 M NH_4OAc/KOH (1:1)	10.0	360	425	0.21

[a] Data obtained from compounds synthesized in solution and purified. [b] External standard: coumarin 120 (ϕ_f=0.88, λ_{ex}=354 nm, λ_{em}=435 nm in EtOH). Error ±10%.

Dependence of the spectral properties of dyes 3e, 3f, 3o, 6c, and 6t on solvent.



compound[a]	R^1	R^2	solvent	λ_{ex}	λ_{em}	ϕ_f[b]
3e	4-OH	4-OMe	$CHCl_3$	333	404	0.66
3e	4-OH	4-OMe	THF	339	405	0.58
3e	4-OH	4-OMe	DMSO	344	440	0.31
3e	4-OH	4-OMe	EtOH	341	424	0.15
3f	4-OH	4-NMe_2	$CHCl_3$	374	471	0.53
3f	4-OH	4-NMe_2	THF	333	486	0.53
3f	4-OH	4-NMe_2	DMSO	341	538	0.17
3f	4-OH	4-NMe_2	EtOH	334	526	0.10
3o	4-OMe	4-OMe	$CHCl_3$	336	393	0.74
3o	4-OMe	4-OMe	THF	337	396	0.72
3o	4-OMe	4-OMe	DMSO	340	419	0.81
3o	4-OMe	4-OMe	EtOH	335	409	0.77
6c	4-OH	4-F	$CHCl_3$	361	417	0.13
6c	4-OH	4-F	THF	361	417	0.12
6c	4-OH	4-F	DMSO	367	454	0.23
6c	4-OH	4-F	EtOH	363	442	0.23
6t	4-OMe	4-F	$CHCl_3$	361	408	0.12
6t	4-OMe	4-F	THF	360	410	0.09
6t	4-OMe	4-F	DMSO	364	429	0.20
6t	4-OMe	4-F	EtOH	361	423	0.22

[a] Data obtained from compounds synthesized in solution and purified. [b] External standard: coumarin 120 (ϕ_f=0.88, λ_{ex}=354 nm, λ_{em}=435 nm in EtOH). Error ±10%.

References and notes.

(1) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*. 2nd ed., New York: Kluwer Academic/Plenum Publishers, 1999.

(2) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923-2925.

(3) For further information about this microwave reactor system, see: www.milestonesci.com/synth-micro.php

(4) (a) Frank, R. *Tetrahedron* **1992**, *48*, 9217-9232. (b) Frank, R. *J. Immunol. Methods* **2002**, *267*, 13-26.

(5) Ma, S.; Venanzi, L. M. *Tetrahedron Lett.* **1993**, *34*, 5269-5272.

(6) (a) Volkmer-Engert, R.; Hoffmann, B.; Schneider-Mergener, J. *Tetrahedron Lett.* **1997**, *38*, 1029-1032. (b) Licha, K.; Bhargava, S.; Rheinlander, C.; Becker, A.; Schneider-Mergener, J.; Volkmer-Engert, R. *Tetrahedron Lett.* **2000**, *41*, 1711-1715.

(7) Ast, T.; Heine, N.; Germeroth, L.; Schneider-Mergener, J.; Wenschuh, H. *Tetrahedron Lett.* **1999**, *40*, 4317-4318.

(8) Carpino, L. A.; Han, G. Y. *J. Org. Chem.* **1972**, *37*, 3404-3409.

(9) The decanted linker solution can be poured onto a second sheet of amino cellulose. Immersion overnight in the solution will give a sheet with similar loadings to the primary sheet.

(10) Scharn, D.; Wenschuh, H.; Reineke, U.; Schneider-Mergener, J.; Germeroth, L. *J. Comb. Chem.* **2000**, *2*, 361-369.

(11) Besson, T; Coudert, G.; Guillaumet, G. *J. Heterocycl. Chem.* **1991**, *75*, 1517-1523.