ELECTRONIC SUPPLEMENTARY INFORMATION

Synthesis and photophysics of novel biocompatible fluorescent oxocines and azocines in aqueous solution.

A. Ulises Acuña[a], Mónica Alvarez-Pérez[a], Marta Liras[b], Pedro B. Coto[c] and Francisco Amat-Guerri[b]*

[a] Instituto de Química Física "Rocasolano", C.S.I.C., Serrano 119, 28006-Madrid, Spain.

[b]Instituto de Química Orgánica General, C.S.I.C., Juan de la Cierva 3, 28006-Madrid, Spain.

[c]Departamento de Química-Física, Universidad de Alcalá de Henares, 28871-Alcalá de Henares, Spain.

Corresponding author:
A. U. Acuña; phone +34 91 5619400, ext. 1220; fax + 34 91 5642431;
e-mail roculises@iqfr.csic.es.

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1. General methods

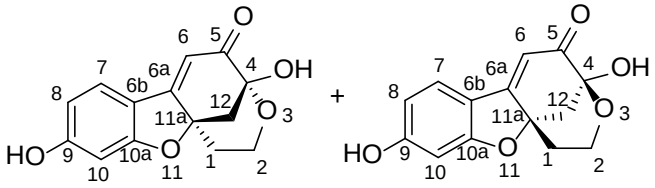
All reagents were obtained from commercial sources and used without further purification unless otherwise stated. Organic solvents of spectroscopic, HPLC and analytical quality were used as required. Water was of MilliQ® quality (Millipore, resistivity 18 MΩ cm). Buffers were prepared by standard procedures.[1] A 414 Crison pH-meter equipped with a 5209 Crison microelectrode was used for pH measurements at room temperature. Analytical thin layer chromatography (TLC) was performed on 0.25 mm thick pre-coated silica gel plates ($60F_{254}$). Semi-preparative thin layer chromatography (TLC) was performed on 0.5 mm thick pre-coated silica gel plates ($60F_{254}$). Column chromatography was performed with polyamide-6 (50-160 μm). Compounds were visualized under UV light exciting at 254 nm and 366 nm. Analytical high performance liquid chromatography (HPLC) analyses for reaction monitoring and purity evaluation were carried out using a photodiode array (PDA) HP Agilent 1100 system equipped with a Eclipse XDB C18 reverse phase column (4.6 × 150 mm, 5 μm) under isocratic elution; in the case of gradient elution a LC-MS Waters system with a 2998 PDA detector and a 3100 mass detector was used, with an analytical SunFire™ reverse phase C18 column (4.6 × 150 mm, 5 μm). Semi-preparative purification was performed in the last system, using a SunFire™ reverse phase C18 column (10 × 150 mm, 5 μm). ^{1}H-NMR and ^{13}C-NMR spectra were recorded in deuterated solvents at 300 or 400 MHz and at 75 or 100 MHz, respectively, using the proton signal of the trace of undeuterated solvent or the carbon signal of the deuterated solvent as internal reference (CD_3OD: 3.31 (*H*), 49.0 (*C*) ppm; CF_3CO_2D: 11.50 (*H*), 116.6, 164.2 (*C*) ppm; D_2O: 4.79 (*H*) ppm; d_7-DMF: 2.74, 2.91, 8.01 (*H*), 30.1, 35.2, 162.7 (*C*) ppm; d_6-DMSO: 2.50 (*H*), 39.5 (*C*) ppm). δ values are reported in ppm and coupling constants are given in Hz. The assignment of chemical shifts is based on standard NMR experiments (^{1}H, ^{13}C-DEPT, ^{1}H, ^{1}H-COSY, gHSQC, gHMBC). Abbreviations: s (singlet), br s (broad singlet), d (doublet), dd (doublet of doublets), ddd (doublet of doublet of doublets), dddd (doublet of doublet of doublet of doublets). IR spectra were recorded on Perkin-Elmer 681 and FT-Spectrum One spectrometers. Low resolution mass spectra were recorded by electron impact (EI) (70 eV) in a Hewlett-Packard 5973 spectrometer in the direct injection mode, or by electrospray ionization (ESI+ or ESI−) in a Hewlett-Packard LC/MS 1100 spectrometer. Elemental analysis was performed with a Heraeus CHN-O-RAPID instrument. Melting points were determined on a Reichert hot-stage microscope and are uncorrected. Optical rotation ($cm^3 \cdot g^{-1} \cdot dm^{-1}$) was measured at 20 ºC on a Perkin-Elmer 241MC polarimeter, using a sodium lamp and a 100 mm path length cuvette. UV-VIS absorption

spectra were registered at room temperature on a Varian CARY-3E spectrophotometer using calibrated 10.00 mm cuvettes. Steady-state fluorescence excitation and emission spectra of optically diluted samples were recorded with a photon-counting spectrofluorometer ISS PC1, U.S.A., and corrected for instrument-dependent factors. Relative fluorescence quantum yields were determined by the comparative method,[2] using as reference that of quinine sulfate in 0.05 M H_2SO_4 (Φ_f = 0.51).[3] Polarized excitation and emission spectra were recorded at 5 ± 1ºC in alkaline glycerol solution (~99.9%) and corrected by standard methods.[4a] Fluorescence lifetimes were recorded by the time-correlated single photon-counting technique with ps time-resolution in a laser spectrometer described elsewhere.[4b] Unless otherwise stated, absorption and fluorescence spectra as a function of pH (pH range 2−12) were recorded using aqueous NaOH or HCl solutions of the required concentration (2−100 mM) and 5 mM Na_2CO_3/$NaHCO_3$ buffer solutions (at pH 9 or 10, depending on the substrate). Fluorometric titrations were carried out using mainly Britton and Robinson´s universal buffer[1] (pH 2.6-12.0).

2. Experimental procedures for the synthesis of oxocines 1 and azocines 2.

General.[5] Equimolar amounts of resorcinol and the catechol derivative in the concentration range 0.5-2×10^{-2} M were dissolved in alkaline water, pH 8.5-11, at room temperature in the presence of air. When required, re-adjustment of the pH was performed by NaOH addition to the reacting mixture. Product reaction monitoring was carried out by TLC, UV-VIS absorption and/or HPLC. For UV-VIS and HPLC analyses, reaction aliquots were acidified (pH 3.5-4) before measurement. When no further reaction was observed, a 1 M HCl or acetic acid (AcH) solution was carefully added until the pH of the mixture was reduced to a value between 3.5 and 7. Additional work-up was then performed according to each product requirements as described below.

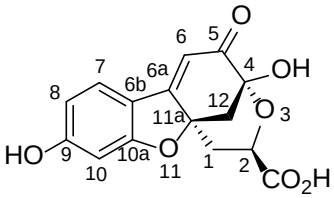
4,9-Dihydroxy-1,2-dihydro-4,11a-methanooxocino[4,5-*b*]benzofuran-5(4*H*)-one (monardine, 1a), (4*R*,11a*R*) and (4*S*,11a*S*) racemic mixture[6]



3-Hydroxytyrosol (2 mmol, 310 mg) and resorcinol (2 mmol, 220 mg) were dissolved in air-saturated 0.2 M NH_4HCO_3 (400 mL, pH = 8.5) and vigorously stirred overnight at room temperature. The reaction mixture was acidified down to pH = 7 (1 M HCl) and extracted with

ethyl acetate (EA). The organic layer was dried (Na_2SO_4) and the solvents evaporated at low pressure. The yellow residue was redissolved in a minimum amount of H_2O and precipitated by addition of EtOH, repeatedly washed with EtOH and once with Et_2O. After vacuum drying, compound **1a** was isolated as a yellow solid (350 mg, 67%), strongly fluorescent in solution. A small fraction was further purified by semi-preparative TLC (DCM-MeOH 96: 4). Mp 196-198 ºC (d). $[\alpha]_D^{20}$ 0 (*c* 0.30 g/100cm^3, MeOH), *racemic*. pK_a 7.2 ± 0.4 (absorption and fluorimetric titration). R_F 0.47 (DCM-MeOH 9: 1). Anal. Calc. for $C_{14}H_{12}O_5$: C, 64.61; H, 4.65. Found: C, 64.39; H, 4.72%. UV-VIS: λ_{max} (H_2O, pH = 4)/nm 305 ± 1 (ε/dm^3·mol^{-1}·cm^{-1} 8680 ± 100) and 382 ± 1 (22930 ± 600). λ_{max} (H_2O, pH = 9)/nm 275 ± 1 (ε/dm^3·mol^{-1}·cm^{-1} 5070 ± 200), 324 ± 1 (2830 ± 200) and 425.5 ± 0.5 (48600 ± 1100). Fluorescence properties: (H_2O, pH = 9) λ_{max} /nm 464± 2; Φ_f 1.0 ± 0.1; τ_f 2.74 ± 0.05 ns. IR: ν_{max} (KBr)/ cm^{-1} 3399, 3191, 1659, 1608, 1581, 1459, 1349, 1177 and 1054. ^{1}H-NMR: δ_H (300 MHz; CD_3OD) 1.59 (1H, ddd, ^2J 12.4, ^3J 3.1, ^4J 2.1, H-1B), 2.22 (1H, ddd, ^2J 12.4, ^3J 12.4, 7.1, H-1A), 2.35 (1H, dd, ^2J 11.1, ^4J 2.1, H-12B), 2.47 (1H, d, ^2J 11.1, H-12A), 3.86 (1H, ddd, ^2J 12.4, ^3J 12.4, 3.1, H-2B), 3.95 (1H, dd, ^2J 12.4, ^3J 7.1, H-2A), 6.32 (1H, s, H-6), 6.39 (1H, d, ^4J 2.0, H-10), 6.56 (1H, dd, ^3J 8.5, ^4J 2.0, H-8), 7.53 (1H, d, ^3J 8.5, H-7). ^{13}C-NMR: δ_C (75 MHz; CD_3OD) 33.4 (CH_2, C-1), 46.5 (CH_2, C-12), 59.9 (CH_2, C-2), 89.6 (C, C-11a), 95.3 (CH, C-10), 99.0 (C, C-4), 111.9 (CH, C-6), 112.9 (CH, C-8), 114.4 (C, C-6b), 126.9 (CH, C-7), 167.0, 167.3, 168.2 (3 × C, C-6a, C-10a, C-9), 194.4 (C, C-5). MS (EI): *m*/*z* 260 (M^+, 45%), 232 (36), 187 (100).

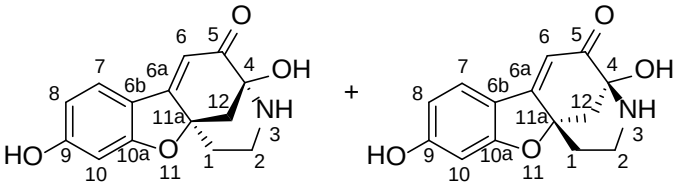
(2*R*,4*R*,11a*R*)-4,9-Dihydroxy-5-oxo-1,2,4,5-tetrahydro-4,11a-methanooxocino[4,5-*b*]benzofuran-2-carboxylic acid (carboxymonardine, 1b)[6]



(2*R*)-3-(3,4-Dihydroxyphenyl)-2-hydroxypropanoic acid (salvianic acid, 0.5 mmol, 99.1 mg) and resorcinol (0.5 mmol, 55.1 mg) were dissolved in air-saturated H_2O (25 mL) adding 0.1 M NaOH up to pH 9.5, and the solution stirred at room temperature for 22 h. The reaction mixture was acidified down to pH 4 (1 M HCl), the solvent evaporated and the residue re-dissolved in H_2O containing 0.1% HCO_2H for semi-preparative HPLC purification (sample concentration: 28 mg·mL^{-1}; injection volume: 1.8 mL; flow rate: 8 mL·min^{-1}; mobile phase A: 0.1% HCO_2H in H_2O, mobile phase B: 0.1% HCO_2H in ACN; gradient profile: 2 to 40% B over 30 min). Collected fractions containing the pure compound (R_t = 16.2 min, strongly

fluorescent in solution) were concentrated *in vacuo* at 25-30 ºC, to yield compound **1b** as a bright yellow solid (68 mg, 45%; estimated conversion by UV-VIS: 57%). Mp 153-155 ºC. $[\alpha]_D^{20}$ -272 (*c* 0.50 g/100cm^3, aqueous 0.1N AcH);[7] $[\alpha]_D^{20}$ -214 (*c* 0.12 g/100cm^3, 0.05 M Na_2CO_3-$NaHCO_3$ buffer, pH = 9). pK_a 7.4 ± 0.4 (absorption and fluorimetric titration). R_F 0.40 ($CHCl_3$-MeOH-AcH 6: 3: 0.5). R_t = 2.64 min (injection volume: 20 µL; flow rate: 1 mL·min^{-1}; mobile phase: AcH-H_2O-MeOH 0.5:79.5:20; λ_{anal} 280, 380 nm). The purity of compound **1b**, as determined by HPLC, was 98-99% (λ 280, 380 nm). UV-VIS: λ_{max} (H_2O, pH = 4)/nm 305 ± 1 (ε/dm^3·mol^{-1}·cm^{-1} 8160 ± 100) and 382 ± 1 (21200 ± 600). λ_{max} (H_2O, pH = 9)/nm 275 ± 1 (ε/dm^3·mol^{-1}·cm^{-1} 4660 ± 200), 324 ± 1 (2810 ± 200) and 425.5 ± 0.5 (44200 ± 300). Fluorescence properties: (H_2O, pH = 9) λ_{max} /nm 464 ± 2; Φ_f 1.0 ± 0.1; τ_f 2.74± 0.05 ns. IR: ν_{max} (KBr)/ cm^{-1} 3412, 1734, 1659, 1606, 1582, 1458, 1357, 1290, 1251, 1180, 1113, 1066, 1017 and 990. ^{1}H-NMR: δ_H (300 MHz; D_2O)[8] 1.94 (1H, dd, ^2J 13.0, ^3J 2.8, H-1B), 2.10 (1H, dd, ^2J 13.0, ^3J 12.5, H-1A), 2.36 (1H, d, ^2J 11.4, H-12B), 2.49 (1H, d, ^2J 11.4, H-12A), 4.36 (1H, dd, ^3J 12.5, ^3J 2.8, H-2), 6.29 (1H, s, H-6), 6.39 (1H, s, H-10), 6.58 (1H, d, ^3J 8.5, H-8), 7.47 (1H, d, ^3J 8.5, H-7). ^{13}C-NMR: δ_C (100 MHz; D_2O) 35.1 (CH_2, C-1), 44.2 (CH_2, C-12), 69.3 (CH, C-2), 88.4 (C, C-11a), 94.5 (C, C-4), 98.4 (CH, C-10), 111.0 (CH, C-6), 112.4 (CH, C-8), 113.6 (C, C-6b), 126.7 (CH, C-7), 164.4 (C, C-9), 166.4 (C, C-10a), 167.2 (C, C-6a), 177.0 (C, CO_2H), 193.3 (C, C-5). MS (ESI$^-$): *m*/*z* 303 (M-H)$^-$.

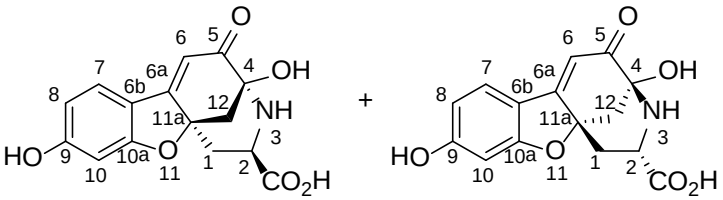
4,9-Dihydroxy-3,4-dihydro-1*H*-4,11a-methanobenzofuro[2,3-*d*]azocin-5(2*H*)-one (azamonardine, 2a), (4*S*,11a*R*) and (4*R*,11a*S*) racemic mixture[6]



3-hidroxytyramine hydrochloride (dopamine hydrochloride, 2 mmol, 379 mg) and resorcinol (2 mmol, 220 mg) were dissolved in air-saturated water (100 mL) adding 1 M NaOH up to pH 11, and the solution stirred at room temperature for 2h. The reaction mixture was acidified down to pH 7 (AcH) and extracted with ethyl acetate (EA). The organic layer was dried (Na_2SO_4) and the solvents evaporated at low pressure. The resulting orange residue was then triturated with water and repeatedly washed with EtOH. After vacuum drying, compound **2a** was obtained as a yellow solid (90 mg, 17 %; estimated conversion by UV-VIS: 19%). Mp >300 ºC (d). $[\alpha]_D^{20}$ 0 (*c* 0.50 g/100cm^3, DMF). p$K_{a\,(C)}$ 5.0 ± 0.4; p$K_{a\,(N)}$ 7.6 ± 0.4 (absorption and fluorimetric titration). R_F 0.46 ($CHCl_3$-MeOH-H_2O 8: 2: 0.5). R_t = 1.80 min (injection

volume: 20 μL; flow rate: 1 mL·min^{-1}; mobile phase: TFA-H_2O-MeOH 0.1: 79.9: 20; λ_{anal} 280, 380 nm). The purity of compound **2a** as determined by HPLC was >98% (λ 280, 380 nm). UV-VIS: λ_{max} (H_2O, pH = 3)/nm 308 ± 1 (ε/dm^3·mol^{-1}·cm^{-1} 8100 ± 400) and 389±1 (25000 ± 600). λ_{max} (H_2O, pH = 10)/nm 275 ± 1 (ε/dm^3·mol^{-1}·cm^{-1} 5000 ± 200), 320 ± 1 (2900 ± 200) and 419 ± 1 (47250 ± 1300). Fluorescence properties: (H_2O, pH = 3) λ_{max} /nm 460 ± 2, Φ_f 0.16 ± 0.03; (H_2O, pH = 10) λ_{max} /nm 462 ± 2, Φ_f 0.47 ± 0.05; intensity weighted average lifetime $<\tau_F>$ = 1.48 ns. IR: ν_{max} (KBr)/ cm^{-1} 3392, 3238, 1654, 1601, 1581, 1494, 1347, 1290 and 1227. ^{1}H-NMR: δ_H (400 MHz; d$_7$-DMF) 1.50 (1H, dddd, ^2J 12.3, ^3J 3.7, ^4J 1.7, ^3J 1.2, H-1B), 1.98 (1H, ddd, ^2J 12.3, ^3J 12.2, ^3J 6.0, H-1A), 2.32 (1H, dd, ^2J 10.4, ^4J 1.7, H-12A), 2.34 (1H, d, ^2J 10.4, H-12A), 2.87 (1H, ddd, ^2J 12.9, ^3J 12.2, ^3J 3.7, H-2B), 2.97 (1H, ddd, ^2J 12.9, ^3J 6.0, ^3J = 1.2, H-2A), 3.54 (2H, br s, NH+OH), 5.65 (1H, br s, OH), 6.34 (1H, s, H-6), 6.50 (1H, d, ^4J 2.0, H-10), 6.62 (dd, ^3J = 8.5 Hz, ^4J = 2.0 Hz, 1H; H-8), 7.67 (1H, d, ^3J 8.5, H-7). ^{13}C-NMR: δ_C (100 MHz; d$_7$-DMF) 32.7 (CH_2, C-1), 37.9 (CH_2, C-2), 47.5 (CH_2, C-12), 83.6 (C, C-4), 90.3 (C, C-11a), 98.6 (CH, C-10), 111.9 (CH, C-8), 112.5 (CH, C-6), 114.0 (C, C-6b), 126.5 (CH, C-7), 164.4 (C, C-6a), 165.7 (C, C-9), 166.7 (C, C-10a), 197.2 (C, C-5). MS (EI): *m*/*z* 259 (M$^+$, 92%), 230 (79), 187 (100).

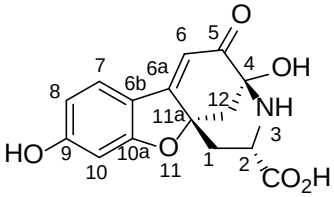
4,9-Dihydroxy-5-oxo-2,3,4,5-tetrahydro-1*H*-4,11a-methanobenzofuro[2,3-*d*]azocine-2-carboxylic acid (carboxyazamonardine, 2b),[9] (2*R*,4*S*,11a*R*) and (2*S*,4*R*,11a*S*) racemic mixture[6]



Racemic 3-(3',4'-dihydroxyphenyl)-alanine (DL-DOPA) (0.48 mmol, 94.6 mg) and resorcinol (0.48 mmol, 52.8 mg) were dissolved in air-saturated Na_2HPO_4-NaOH buffer (120 mL, 0.25 M, pH 11), and the solution stirred at room temperature for 28h. The reaction mixture was acidified down to pH 4 (AcH), the precipitate formed collected and thoroughly washed with aqueous 0.1 M AcH. After vacuum drying, compound **2b** was isolated as a beige solid (56 mg, 39%; estimated conversion by UV-VIS: 81%). Mp >300 ºC. p$K_{a\,(C)}$ 5.0 ± 0.4; p$K_{a\,(N)}$ 7.6 ± 0.4 (absorption and fluorimetric titration). R_F 0.14 ($CHCl_3$-MeOH-AcH 6: 3: 0.5). R_t = 6.82 min (injection volume: 20 μL; flow rate: 1 mL·min^{-1}; mobile phase: aqueous 0.1 M AcH; λ_{anal} 280, 380 nm). The purity of compound **2b** as determined by HPLC was >99% (λ 280, 380 nm). UV-VIS: λ_{max} (H_2O, pH = 3)/nm 308 ± 1 (ε/dm^3·mol^{-1}·cm^{-1} 7820 ± 400) and 389 ± 1 (24700 ±

600). λ_{max} (H_2O, pH = 10)/nm 275 ± 1 (ε/dm^3·mol^{-1}·cm^{-1} 5700 ± 200), 320 ± 1 (3010 ± 200) and 419 ± 1 (44680 ± 1300). Fluorescence properties: (H_2O, pH = 3) λ_{max} /nm 460 ± 2, Φ_f 0.25 ± 0.03; (H_2O, pH = 10) λ_{max} /nm 462 ± 2, Φ_f 0.45 ± 0.05; intensity weighted average lifetime $<\tau_F>$ = 1.24 ns. IR: ν_{max} (KBr)/ cm^{-1} 3231, 3054, 2916, 2768, 2530, 2444, 1671, 1644, 1628, 1585, 1564, 1456, 1405, 1358, 1311, 1295, 1244, 1220, 1165, 1061, 1004 and 810. ^{1}H-NMR: δ_H (400 MHz; d$_6$-DMSO-0.1% CF_3CO_2D) 1.73 (1H, dd, ^2J 12.1, ^3J 3.4, H-1B), 1.90 (1H, dd, ^2J 12.1, ^3J 12.0, H-1A), 2.22 (1H, d, ^2J 10.6, H-12B), 2.29 (1H, d, ^2J 10.6, H-12A), 3.50 (1H, dd, ^3J 12.0, ^3J 3.4, H-2), 3.86 (2H, broad s, NH+OH), 6.04 (1H, broad s, OH), 6.33 (1H, s, H-6), 6.43 (1H, d, ^4J 1.4, H-10), 6.55 (1H, dd, ^3J 8.4, ^4J 1.4, H-8), 7.62 (1H, d, ^3J 8.4, H-7), 10.66 (1H, br s, CO_2H). ^{13}C-NMR: δ_C (100 MHz; d$_6$-DMSO-0.1% CF_3CO_2D) 34.7 (CH_2, C-1), 45.8 (CH_2, C-12), 50.0 (CH, C-2), 82.4 (C, C-4), 88.5 (C, C-11a), 97.9 (CH, C-10), 111.4 (CH, C-8), 112.1 (CH, C-6), 112.7 (C, C-6b), 126.1 (CH, C-7), 162.9 (C, C-6a), 164.6, 165.5 (2 × C, C-10a, C-9), 172.4 (C, CO_2H), 195.2 (C, C-5). MS (ESI$^+$): *m*/*z* 304 $(M+H)^+$.

(2*S*,4*R*,11a*S*)-4,9-Dihydroxy-5-oxo-2,3,4,5-tetrahydro-1*H*-4,11a-methanobenzofuro[2,3-*d*]azocine-2-carboxylic acid [(2*S*,4*R*,11a*S*)-2b].

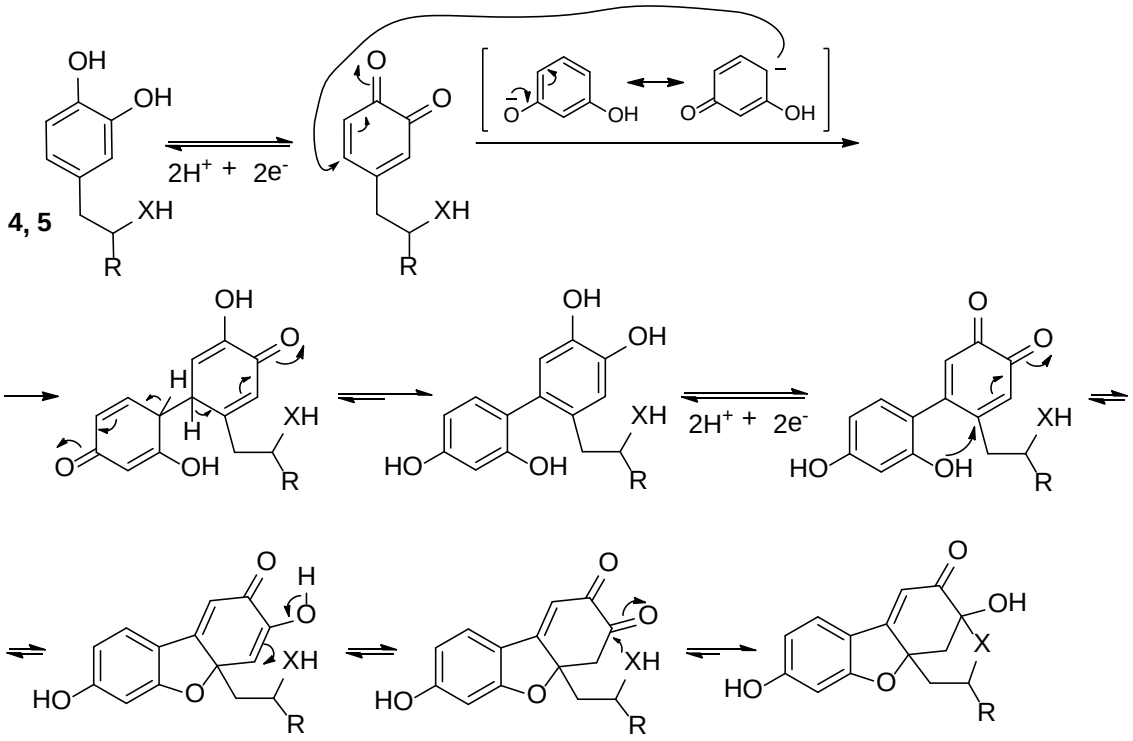


Optically active carboxyazamonardine was obtained by the same procedure described above, but using 3-(3',4'-dihydroxyphenyl)-L-alanine (L-DOPA) as reactant, $[\alpha]_D^{26}$ +287 (*c* 0.10 g/100cm^3, DMSO-0.1%TFA) : 54 mg, 37%; estimated conversion by UV-VIS: 78%. All the spectral data compared well to the values registered for the racemic product. Purity was determined by HPLC as >99% (λ 280, 380 nm).

3. Miscellaneous reaction conditions

It is well knonw that catechol oxidation is strongly dependent on molecular oxygen concentration and solution pH,[10] among other factors. Therefore, a number of exploratory experiments were carried out adjusting these factors to optimize the rate and yield of the fluorogenic reaction. Catechol oxidation, first to semiquinone and then to the corresponding quinone, is required for the formation of the fluorescent compounds reported here, presumably through the reaction mechanism shown in Scheme S1, which is based on that postulated

before for the *intra*molecular oxidative coupling of coatline B that produced a glucosilated analogue of **1b**.[6] A crucial initial reaction step in this scheme would be the nucleophilic attack of resocinol monoanion to the quinone species.



Scheme S1. A plausible mechanism of the fluorogenic reaction of catechols and aminocatechols (X = O, NH; R = H, CO_2H) with resorcinol.

The effect of resorcinol properties, as charge distribution and pKa, on the oxidative coupling reaction was investigated using three additional resorcinol derivatives: 5-methylresorcinol, 5-fluororesorcinol and 5-hydroxyresorcinol. All three compounds yielded the fluorogenic reaction and no large changes in reaction rate could be observed. Thus, 5-methylresorcinol behaved quite similarly to resorcinol, 5-hydroxyresorcinol give rise to a more complex mixture of reaction products and 5-fluororesorcinol decreased both reaction rate and yield. In the last case, it is likely that the fluoro substituent may impair the nucleophilic reactivity against the quinone intermediate.

Regarding the effect of oxygen it was observed that the open-air reaction was faster in solutions containing a catechol concentration less or equal to 10^{-4} M, which is close to the oxygen equilibrium concentration in water at room temperature and normal pressure; vigorous stirring in these conditions favored the reaction clearly. On the other hand, an excess of resorcinol increased the reaction rate only when the concentration of the catechol was, at least, in the range of the dissolved oxygen concentration. When catechol concentration was higher

than oxygen concentration, a ten-fold excess of resorcinol depressed the reaction rate, presumably due to competitive oxidation of resorcinol[11].

Catechol oxidation was also accelerated by adding to the reaction solution a small amount of hydrogen peroxide, either directly or as an urea complex (UHP), and catalase. Several relative concentrations of these compounds were tested but with little success because the fluorescent reaction products were unstable in these reaction conditions.

Reaction rates increased with increasing pH, and all these compounds required a pH ≥ 8 to react in convenient times. On the other hand, the effect of solution alkalinity was different for the catechols and aminocatechols. Thus, dopamine and DOPA reaction required higher solution pH than that of hydroxytyrosol and salvianic acid. At pH 9, the reactivity varied as hydroxytyrosol > salvianic acid > dopamine > DOPA. Such differential reactivity may be due to the corresponding variation of the catechol oxidation potential with pH.[12]

A variety of buffer compositions were tested to set the desired reaction pH. Solutions based on NH_4HCO_3 were a convenient choice for the fluorogenic reaction of hydroxytyrosol or salvianic acid, which occurred at a substantial rate at pH 8.5. Since this buffer is volatile, it may be an interesting option for large-scale preparation of monardine (**1a)** and carboxymonardine (**1b)**. Na_2CO_3-$NaHCO_3$ and Na_2HPO_4-NaOH solutions are appropriate media when working at pH 9-10 or pH 11 values; occasionally, plain NaOH could be used with similar results. Buffers based on glycine-NaOH or borax-NaOH (pH 8.5-9) were of less utility. In the case of borax, this was not unexpected, since boronic compounds stabilize catechols against oxidation.[13]

It is also well known that catechol oxidation can be accelerated enzymatically or by heavy metals.[12b] We observed that using $CuSO_4$ (one equivalent) or the copper-containing enzyme tyrosinase increased the oxidation step rate (as detected by UV-VIS spectra). However, this was accompanied by a lower yield of the fluorescent product and the formation of dark polymeric side-products, likely derived from quinone self-coupling. Presumably, quinone intermediates were produce much faster than could be captured by resorcinol.

Finally, a moderate rise in reaction temperature decreased reaction times but more secondary products were produced. Thus, room temperature was selected for all the reactions shown here.

4. Figures S1 and S2

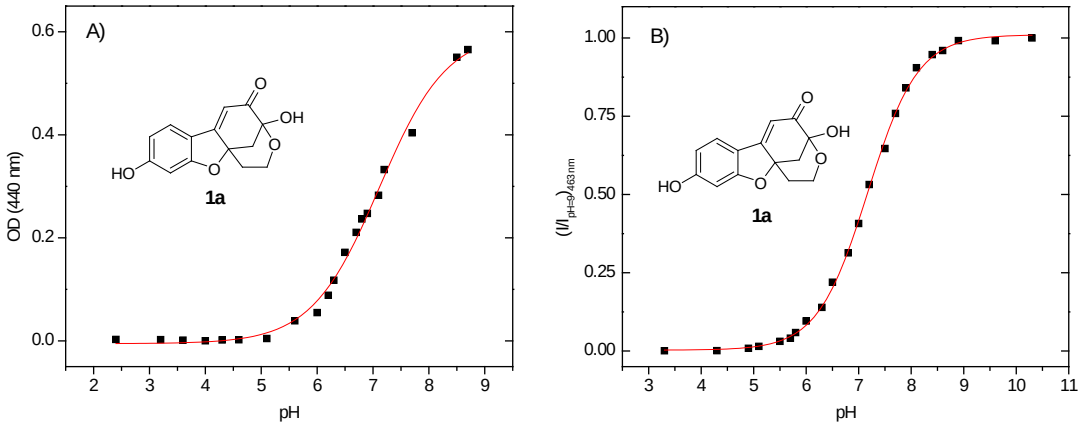


Figure S1. Sample titration curves for the determination of pKa value of monardine (**1a**). A) Absorption titration at 440 nm and B) emission titration, λ_{exc} = 425 nm, λ_{em} = 460 nm. The absorption titration was started from a basic solution (NaOH, pH 9) by adding a solution of HCl. The fluorimetric titration was started from an acidic solution (universal buffer[1]) by adding NaOH. The pK_a values obtained from fitting a sigmoidal Boltzmann function (─) to these data were 7.5 ± 0.4 (absorption) and 7.4 ± 0.4 (fluorescence). NaOH/HCl solutions are more convenient (less absorbing) that the universal buffer for recording absorption spectra in an extended UV range.

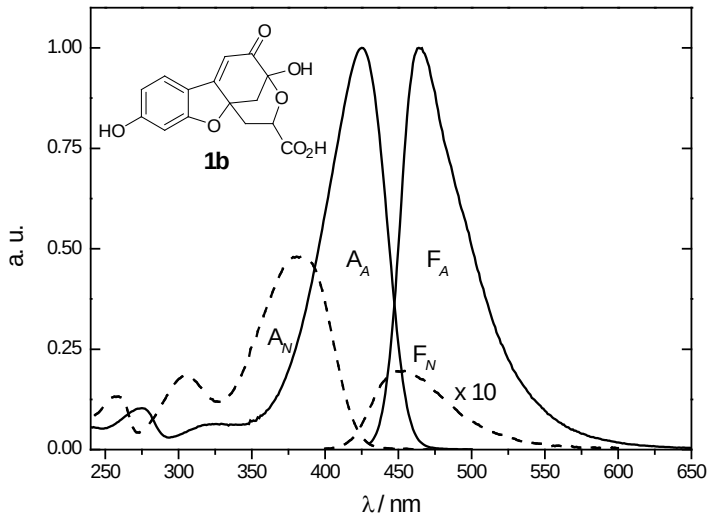


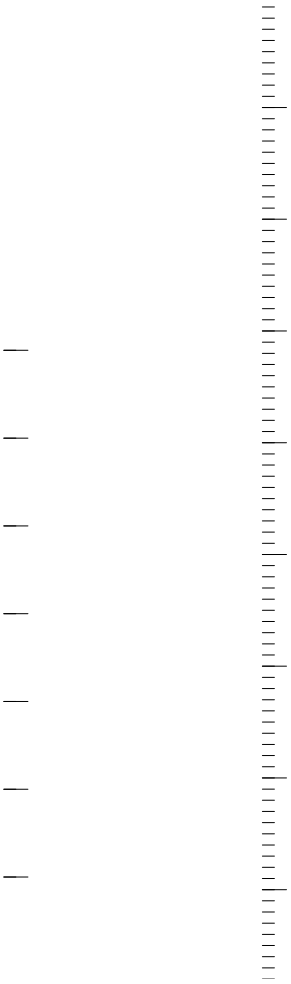
Figure S2. Absorption and corrected fluorescence spectra of the neutral and anionic forms of carboxymonardine, **1b,** in aqueous solution. $\mathbf{A_N}$ and $\mathbf{F_N}$: Absorption and fluorescence spectra of the neutral form at pH 4. $\mathbf{A_A}$ and $\mathbf{F_A}$: absorption and fluorescence spectra of the anion from the same solution at pH 9. [CMO] =1.6 x 10^{-6} M, λ_{exc}= 425 nm ± 2 nm.

5. NMR spectra of products

Compound 1a, CD_3OD, ^{1}H-NMR, 300 MHz

Compound 1a, CD_3OD, ^{13}C-NMR, 300 MHz

Compound 1b, d_6-DMSO, ^{1}H-NMR, 300 MHz



Compound 1b, D_2O, ^{1}H-NMR, 300 MHz

Compound 1b, D_2O, ^{13}C-NMR, 100 MHz

Compound 2a, d_7-DMF, ^{1}H-NMR, 400 MHz

Compound 2a, d_7-DMF, ^{13}C-NMR, 100 MHz

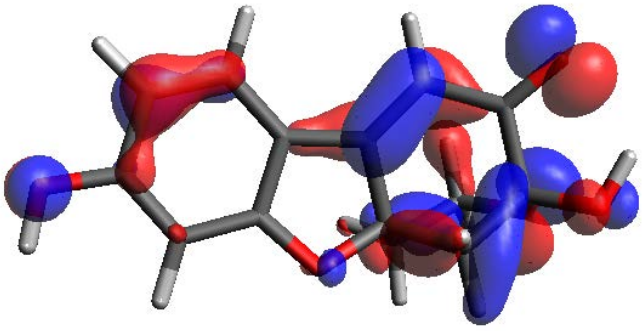
Compound 2b, d_6-DMSO-0.1% CF_3CO_2D, ^{1}H-NMR, 400 MHz

Compound 2b, d_6-DMSO-0.1% CF_3CO_2D, ^{13}C-NMR, 100 MHz

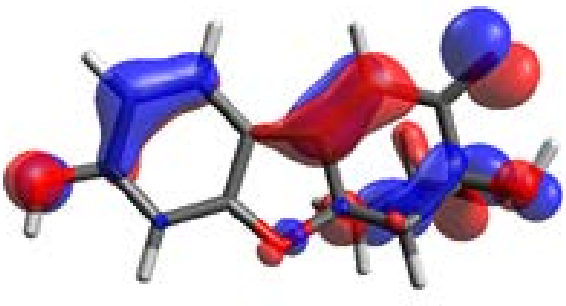
6. Computational details

The systems investigated in this work have been modeled using density functional theory (DFT); ground state equilibrium structures and vertical excitation energies of the different molecules were computed using the HSE06 functional. Vertical excitation energies were compared with experimental data corresponding to absorption maxima, assuming that the uncertainty introduced in that way would be lower than that of the computational method. This functional was not specifically designed for charge-transfer states and can be problematic in the description of excited states with strong charge-transfer character, underestimating the relative energy of these states. Nevertheless, in the case of the ionized species studied in the present work, the overall computed excitation energies obtained with this functional agree quite well with the experimental data, keeping in mind that some of these energies for non-emitting states are underestimated. On the other hand, the application of the HSE06 functional in the particular case of azamonardine zwitterion is more questionable. Therefore, the results obtained with the HSE06 functional for this species were compared with those obtained with the CAM-B3LYP[14] and LC-wPBE[15] functionals. It was found that i) for the lowest-lying S_1 state the three functionals render similar excitation energies (3.2 eV, 3.3 eV and 3.3 eV, respectively) and state character, and ii) for higher excited states discrepancies were much important. In fact, computations with the HSE06 functional produced some low-energy singlet excited states which are not present for the rest of the functionals, and that could be over-stabilized due to the charge-transfer character.

Figure S3. Monardine**:** molecular orbitals involved in the transitions of the five lowest-lying singlet excited states in water solution.

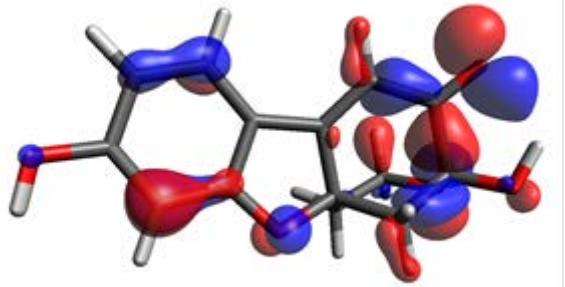


HOMO-4



HOMO-3

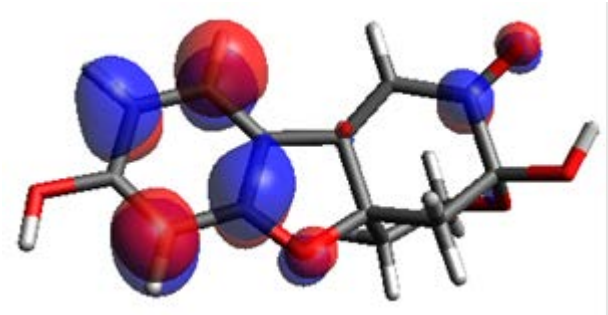
HOMO-2



HOMO-1

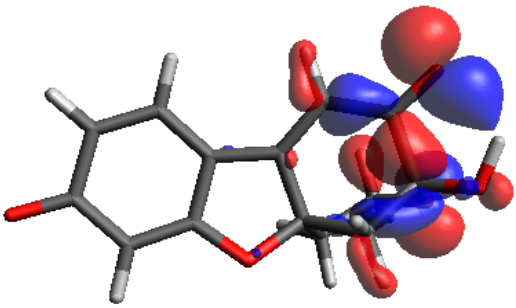
HOMO

LUMO

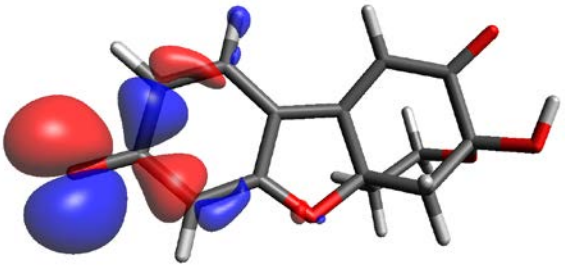


LUMO+1

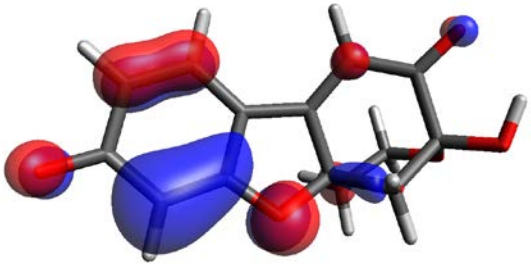
Figure S4. Monardine anion: molecular orbitals involved in the transitions of the five lowest-lying singlet excited states in water solution.



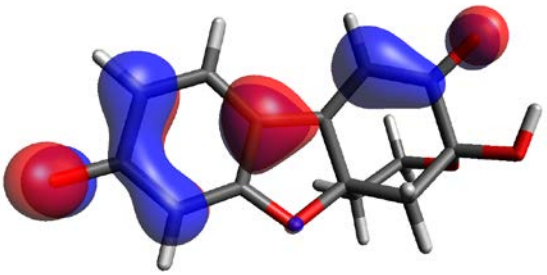
HOMO-3



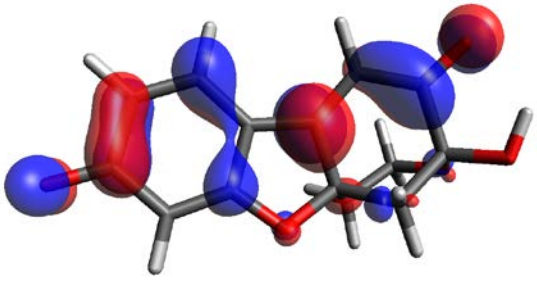
HOMO-2



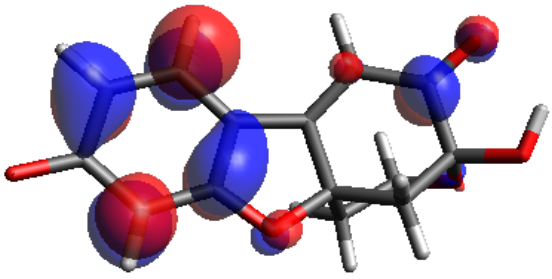
HOMO-1



HOMO

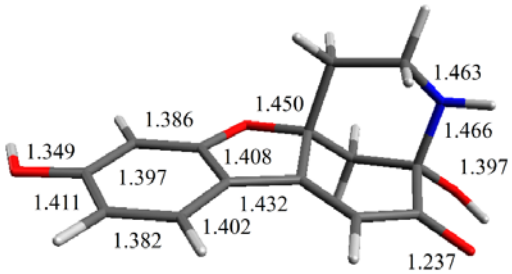


LUMO

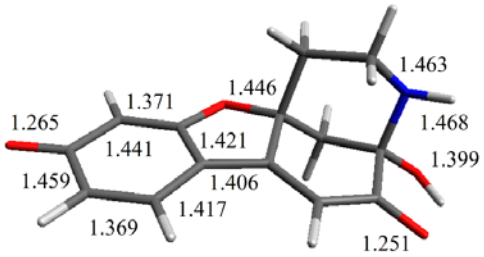


LUMO+1

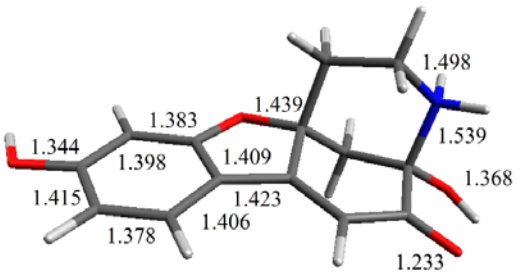
Figure S5. Most significant computed bond distances of azamonardine neutral (N), anionic (A) and cationic (C) forms.



N

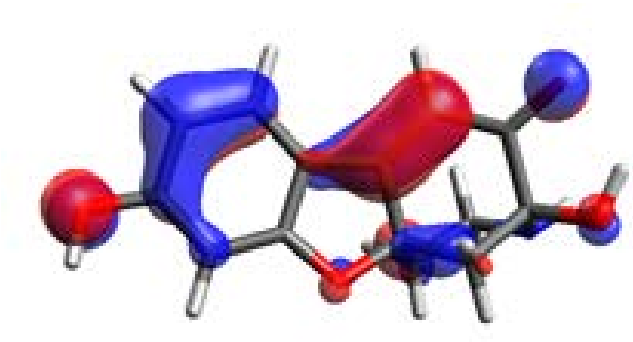


A

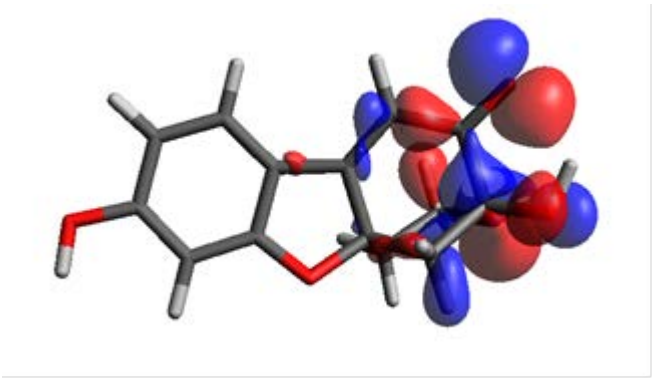


C

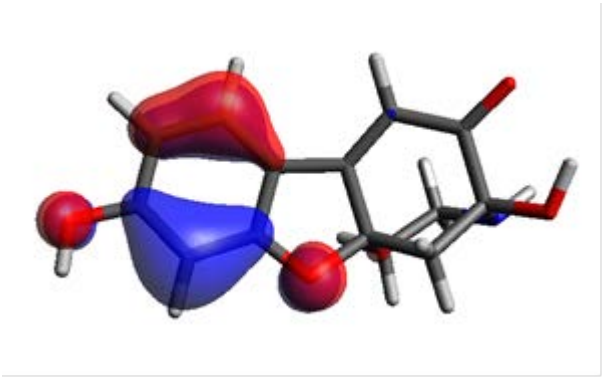
Figure S6. Azamonardine: molecular orbitals involved in the transitions of the five lowest-lying singlet excited states in water solution.



HOMO-4

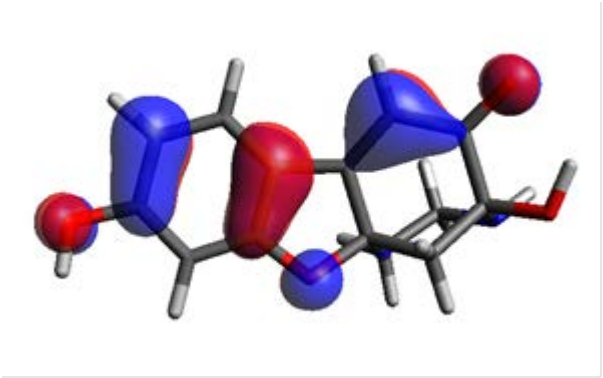


HOMO-3

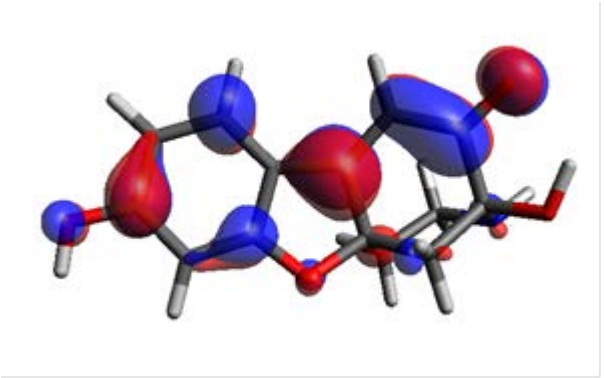


HOMO-2

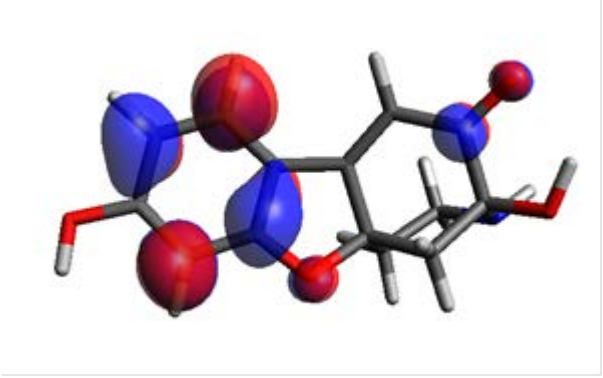
HOMO-1



HOMO

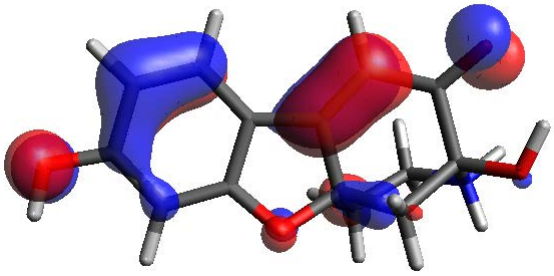


LUMO

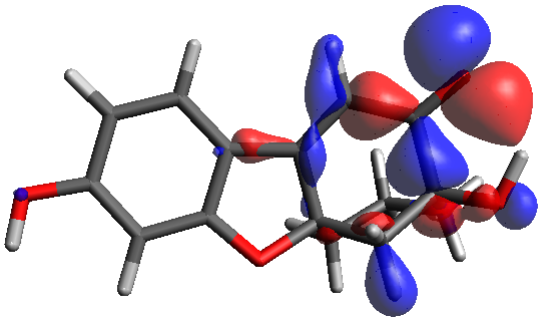


LUMO+1

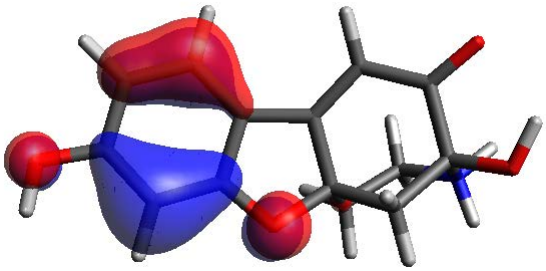
Figure S7. Azamonardine cation: molecular orbitals involved in the transitions of the five lowest-lying singlet excited states in water solution.



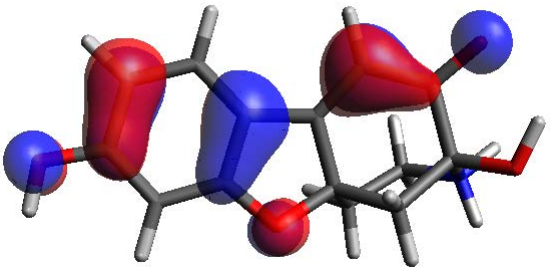
HOMO-3



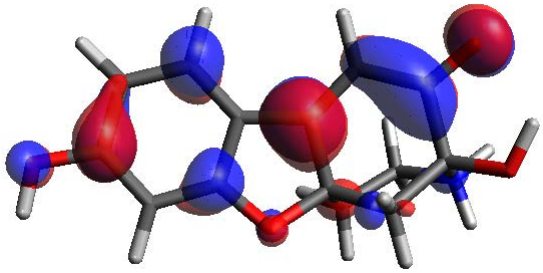
HOMO-2



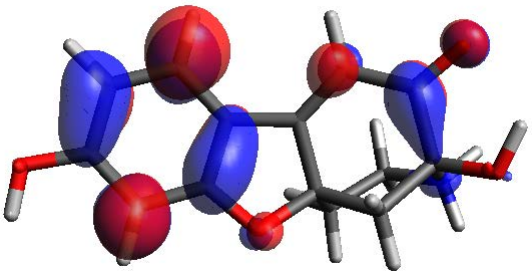
HOMO-1



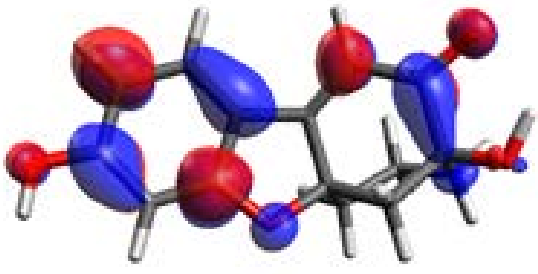
HOMO



LUMO

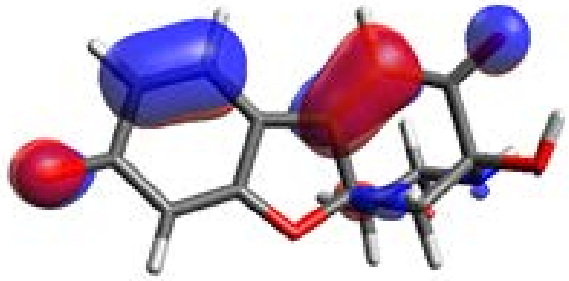


LUMO+1



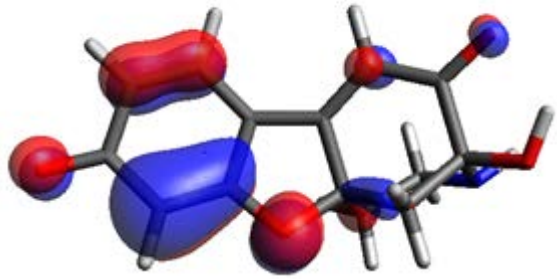
LUMO+2

Figure S8. Azamonardine anion: molecular orbitals involved in the transitions of the five lowest-lying singlet excited states in water solution.



HOMO-4

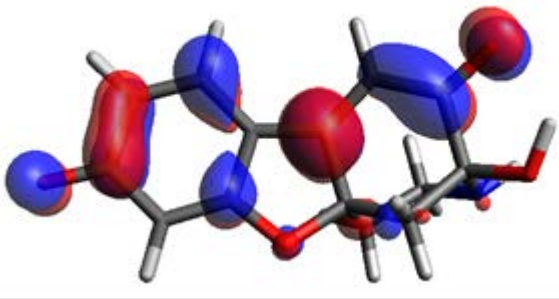
HOMO-3



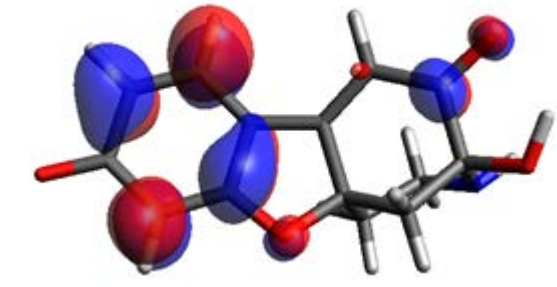
HOMO-2

HOMO-1

HOMO



LUMO



LUMO+1

Table S1. Transition dipole moment components (a.u.) of the five lowest-lying singlet excited states of monardine and its anion in water solution, computed at the LR-TDDFT level of theory[a].

State	Monardine			Monardine anion		
	x	y	z	x	y	z
S1	2.0776	-0.6147	-0.0211	3.4567	-0.4672	-0.0458
S2	1.2510	-0.3675	-0.0830	-0.0136	0.0005	0.0047
S3	1.5560	0.1054	0.0556	0.2356	0.3205	-0.0288
S4	-0.0877	0.0357	-0.0277	0.1941	-0.1879	-0.0513
S5	-0.3529	-0.4267	-0.0765	0.2467	-0.5974	-0.1334

[a] HSE06/6-311++G(d,p)//HSE06/6-31++G(d,p)

Table S2. Transition dipole moment components (a.u.) of the five lowest-lying singlet excited states of neutral, cationic and anionic azamonardine in water solution, computed at the LR-TDDFT level of theory [a].

State	Azamonardine			Azamonardine cation			Azamonardine anion		
	x	y	z	x	y	z	x	y	z
S1	0.2695	0.1249	-0.1145	-2.4809	0.7154	0.0521	3.4472	-0.4437	-0.0520
S2	2.4109	-0.6831	-0.0613	1.3362	0.1083	0.0884	0.0053	0.1560	-0.0937
S3	1.4939	0.1364	0.0718	-1.0093	0.0188	-0.0041	-0.0486	-0.0172	0.0181
S4	-0.2818	0.1586	-0.0537	0.3412	0.1024	0.0775	0.1317	0.3648	0.0128
S5	-0.3562	-0.4800	-0.1102	-0.0132	0.6077	-0.0480	0.2720	-0.6163	-0.1745

[a] HSE06/6-311++G(d,p)//HSE06/6-31++G(d,p)

Table S3. Most significant singlet excitation contribution to the five lowest-lying triplet excited states of monardine and its anion in water solution, computed at the LR-TDDFT level of theory[a].

State	Monardine	Monardine anion
T1	HOMO→LUMO (0.68)	HOMO→LUMO (0.71)
T2	HOMO-1→LUMO (0.61)	HOMO-1→LUMO (0.67)
T3	HOMO-2→LUMO (0.57)	HOMO-2→LUMO (0.68)
T4	HOMO-3→LUMO (0.49)	HOMO-3→LUMO (0.46)
T5	HOMO→LUMO+1 (0.44)	HOMO-3→LUMO (0.48)

[a] HSE06/6-311++G(d,p)//HSE06/6-31++G(d,p)

Table S4. Relative energies (eV) of the five lowest-lying triplet states of neutral, cationic and anionic azamonardine computed at the LR-TDDFT level of theory in water solution[a].

State	Azamonardine	Azamonardine cation	Azamonardine anion
T1	2.27	2.13	1.94
T2	2.91	3.21	3.30
T3	3.40	3.50	3.34
T4	3.89	3.77	3.42
T5	4.01	4.03	3.55

[a] HSE06/6-311++G(d,p)//HSE06/6-31++G(d,p)

Table S5. Most significant singlet excitation contribution to the five lowest-lying triplet states of neutral, cationic and anionic azamonardine, computed at the LR-TDDFT level of theory in water solution.[a]

State	Azamonardine	Azamonardine cation	Azamonardine anion
T1	HOMO→LUMO (0.68)	HOMO→LUMO (0.69)	HOMO→LUMO (0.71)
T2	HOMO-1→LUMO (0.66)	HOMO-1→LUMO (0.65)	HOMO-1→LUMO (0.60)
T3	HOMO-2→LUMO (0.62)	HOMO-2→LUMO (0.67)	HOMO-2→LUMO (0.61)
T4	HOMO-4→LUMO (0.57)	HOMO-3→LUMO (0.61)	HOMO-3→LUMO (0.68)
T5	HOMO→LUMO+1 (0.48)	HOMO→LUMO+2 (0.41)	HOMO-4→LUMO (0.54)

[a] HSE06/6-311++G(d,p)//HSE06/6-31++G(d,p)

Table S6. Cartesian coordinates (in angstrom) for all the systems investigated in this work. The geometry was optimized in solution using the PCM model and the HSE06/6-31++G(d,p) level of theory.

	Monardine				Monardine anion		
	x	y	z		x	y	z
C	3.669166	1.317719	0.316195	C	3.651207	1.346070	0.263125
C	4.065291	0.040850	-0.138488	C	4.128188	0.035600	-0.169311
C	3.136043	-0.959501	-0.436675	C	3.130587	-0.970374	-0.434198
C	1.799898	-0.636918	-0.260829	C	1.809792	-0.651168	-0.258386
C	1.374382	0.625572	0.193780	C	1.356433	0.625339	0.174740
C	2.329736	1.613702	0.475384	C	2.322590	1.633432	0.421428
C	-0.441142	-0.836722	-0.085024	C	-0.438573	-0.846975	-0.069410
C	-1.549362	-0.903113	-1.119762	C	-1.554027	-0.915569	-1.097453
C	-2.726309	-0.154541	-0.501414	C	-2.726039	-0.162357	-0.476728
C	-2.353495	1.305852	-0.110861	C	-2.354617	1.298061	-0.087190
C	-0.989401	1.596368	0.237381	C	-1.010487	1.588128	0.253638
C	-0.054926	0.604864	0.166728	C	-0.046904	0.597291	0.166956
C	-0.901862	-1.548961	1.193680	C	-0.899933	-1.552356	1.213244
C	-2.205820	-0.959389	1.707989	C	-2.206483	-0.970495	1.729881
O	0.778381	-1.478669	-0.528661	O	0.773041	-1.496633	-0.510394
O	5.392500	-0.157761	-0.268055	O	5.364568	-0.190836	-0.304602
O	-3.261550	2.141392	-0.153580	O	-3.291322	2.122561	-0.134864
O	-3.178511	-0.834946	0.671658	O	-3.179769	-0.852899	0.694989
O	-3.787437	-0.117741	-1.378745	O	-3.796102	-0.116279	-1.346518
H	5.575632	-1.050902	-0.586528	H	4.409245	2.100140	0.457350
H	4.437950	2.051381	0.532307	H	3.441954	-1.952441	-0.776156
H	3.442429	-1.938563	-0.790565	H	2.005578	2.622517	0.743009
H	2.024139	2.596518	0.820133	H	-0.741053	2.619849	0.463015
H	-0.720651	2.628090	0.444270	H	-0.126364	-1.472557	1.984237
H	-0.131011	-1.476052	1.967755	H	-1.029243	-2.612490	0.967382
H	-1.034909	-2.606367	0.940904	H	-4.243929	0.725834	-1.130739
H	-4.282423	0.691748	-1.155368	H	-1.252374	-0.431019	-2.030763
H	-1.243352	-0.415543	-2.049693	H	-1.851770	-1.948862	-1.304497
H	-1.845579	-1.935481	-1.330007	H	-2.649439	-1.627041	2.481855
H	-2.652636	-1.614445	2.458093	H	-2.033369	0.008240	2.196621
H	-2.029849	0.016732	2.179249				

Azamonardine

	x	y	z
C	3.658446	1.322989	0.310678
C	4.056652	0.050486	-0.152188
C	3.128918	-0.949761	-0.455163
C	1.791804	-0.632156	-0.274478
C	1.364462	0.626316	0.188865
C	2.317697	1.614507	0.473871
C	-0.447685	-0.842474	-0.081007
C	-1.571362	-0.919616	-1.097381
C	-2.748711	-0.167718	-0.478541
C	-2.363380	1.300060	-0.154546
C	-1.002668	1.590611	0.217959
C	-0.066733	0.601435	0.162045
C	-0.866827	-1.555357	1.212970
C	-2.142225	-0.956606	1.786008
N	-3.162412	-0.866855	0.741367
O	0.771964	-1.474645	-0.546203
O	5.384885	-0.145613	-0.285126
O	-3.253986	2.154557	-0.243757
O	-3.827076	-0.139631	-1.365594
H	5.567843	-1.036990	-0.608474
H	4.425482	2.057446	0.530488
H	3.437209	-1.925578	-0.816415
H	2.010541	2.594563	0.825263
H	-0.735494	2.625689	0.410771
H	-0.058356	-1.495499	1.948276
H	-1.021311	-2.609857	0.958541
H	-4.236329	0.736662	-1.243570
H	-1.278375	-0.441450	-2.036553
H	-1.866359	-1.955231	-1.292540
H	-2.524906	-1.601156	2.582116
H	-1.913516	0.018509	2.244354
H	-3.999803	-0.417862	1.103422

Azamonardine cation

	x	y	z
C	3.647837	1.327503	0.296469
C	4.046768	0.049968	-0.162023
C	3.120712	-0.956021	-0.454161
C	1.787613	-0.637599	-0.266583
C	1.358092	0.624395	0.190213
C	2.311701	1.619849	0.464720
C	-0.443912	-0.847063	-0.072149
C	-1.559361	-0.931623	-1.102376
C	-2.731799	-0.148197	-0.534338
C	-2.356840	1.309931	-0.127350
C	-1.004397	1.591572	0.233538
C	-0.064757	0.598677	0.168551
C	-0.869043	-1.564656	1.219936
C	-2.144551	-0.996428	1.809458
N	-3.192580	-0.866039	0.746499
O	0.764833	-1.483840	-0.525362
O	5.370230	-0.139208	-0.299831
O	-3.276879	2.127693	-0.205972
O	-3.804010	-0.128619	-1.383503
H	5.560980	-1.030783	-0.619439
H	4.416639	2.062552	0.506960
H	3.428425	-1.932811	-0.812082
H	2.004154	2.600850	0.811886
H	-0.736948	2.623891	0.434387
H	-0.076803	-1.487413	1.969417
H	-0.993837	-2.624717	0.976155
H	-4.236131	0.740040	-1.254277
H	-1.258127	-0.461414	-2.041762
H	-1.847217	-1.968335	-1.308727
H	-2.548322	-1.641366	2.590127
H	-1.994844	-0.000401	2.229111
H	-3.534426	-1.789287	0.461856
H	-4.009466	-0.372496	1.119126

Azamonardine anion

	x	y	z
C	3.651221	1.345725	0.284094
C	4.128276	0.039988	-0.159172
C	3.130310	-0.961729	-0.435474
C	1.808231	-0.643447	-0.259710
C	1.355356	0.628568	0.184129
C	2.321397	1.632650	0.441482
C	-0.440478	-0.842469	-0.068896
C	-1.562657	-0.911535	-1.088941
C	-2.738794	-0.158977	-0.470434
C	-2.353314	1.306354	-0.126942
C	-1.012255	1.592357	0.241446
C	-0.050361	0.601646	0.168487
C	-0.875099	-1.553542	1.221459
C	-2.158085	-0.964235	1.787648
N	-3.170222	-0.877806	0.734976
O	0.772035	-1.485762	-0.523939
O	5.365933	-0.186782	-0.294236
O	-3.272857	2.150152	-0.208795
O	-3.815616	-0.108534	-1.362601
H	4.408766	2.097880	0.487884
H	3.441176	-1.940966	-0.786247

H	2.004885	2.619295	0.771292
H	-0.742975	2.625850	0.443306
H	-0.071553	-1.491705	1.962336
H	-1.024559	-2.609469	0.967386
H	-4.194805	0.781850	-1.225122
H	-1.265229	-0.428769	-2.024737
H	-1.858687	-1.946013	-1.292266
H	-2.543231	-1.611868	2.580782
H	-1.937627	0.013198	2.245231
H	-4.006157	-0.421917	1.092345

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5. The experimental procedures detailed here are appropriate to prepare a fraction of gram of the target compound, enough to fully characterize these novel fluorophores. In Section 3, a variety of alternative reaction conditions are summarized which were designed to optimize reaction conditions.

6. The stereochemical configuration was assigned based on our previous work: A. U. Acuña, F. Amat-Guerri, P. Morcillo, M. Liras, B. Rodriguez *Org. Lett.* **2009**, *11*, 3020-3023.

7. Starting salvianic acid ((*R*)-a,3,4-trihydroxy-benzenepropanoic acid): $[\alpha]^{20}_{D}$ = +21.5 (c = 0.52 g/100cm^3, aqueous 0.1M AcH).

8. ^{1}H-NMR in d_6-DMSO was similar to that reported in ref. 6.

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