Electronic Supplementary Information

Water-soluble, pH-sensitive fluorescent probes on the basis of acridizinium ions

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Materials and methods. The 9-carboxyacridizinium bromide and *N*-(3-dimethylaminopropyl)-acridizinium-9-carboxamide were synthesized according to published procedures.[1] Solvents employed in spectroscopic experiments were of spectral grade; purified water with resistivity ≥ 18 MΩ cm^{-1} was used for the preparation of the buffer solutions.

Spectrophotometric measurements. Absorption spectra were recorded with a double-beam spectrophotometer; corrected fluorescence emission spectra were recorded at a right-angle sample orientation in quartz sample cells in spectral-grade solvents. Working solutions were prepared directly prior to use by dilution of corresponding stock solutions (1.00 mM in MeOH or water) to the desired concentrations. The relative fluorescence quantum yields, ϕ_f, were determined by standard methods[2] relative to Coumarin 153 (ϕ_{fl} = 0.38 in EtOH) or Coumarin 1 (ϕ_{fl} = 0.73 in EtOH).[3]

The solubilty of compound **3** was determined from a saturated solution in Britton-Robinson buffer at pH = 7.0. The solution was diluted stepwise to a concentration of ca. 10^{-4} M, and subsequently the concentration was determined by photometric analysis.

Spectrophotometric titrations of acridizinium derivative 3. The Britton–Robinson buffer solution was prepared from phosphoric acid, boric acid, and sodium acetate (0.04 M each) in water and adjusted to pH = 7.0 by the addition of aq. solution of NaOH (2 M). Compound **3** was dissolved in the buffer solution (c = 1.0 × 10^{-4} M), and subsequently aliquots of aq. HCl (2 M) were added. After each addition step, the pH and the absorption spectra were determined with a sample of ca. 3 ml. After recording each absorption spectrum, the sample was quantitatively returned to the titration beaker. The titrations were performed three times in the pH range between 7 and 1, and the data from the absorption spectra were plotted as function of pH of the solution. The value of the acidity

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[1] (a) C. K. Bradsher, and J. C. Parham, *J. Heterocycl. Chem.*, 1964, **1**, 30. (b) K. Benner, A. Granzhan, H. Ihmels, and G. Viola, *Eur. J. Org. Chem.,* 2007, 4721.
[2] (a) Valeur, B. *Molecular Fluorescence: Principles and Applications*, Wiley–VCH, Weinheim, **2002**. (b) Demas, J. N.; Crosby, G. A. *J. Phys. Chem.* **1971**, *75*, 991.
[3] Jones, G.; Jackson, W. R.; Choi, C. Y.; Bergmark, W. R. *J. Phys. Chem*. **1985**, *89*, 294.

constant pK_a was obtained by numerical fitting of the experimental data to the Henderson–Hasselbalch equation in the form of eq S1.[4]

$$A = \frac{A_{HA}\, 10^{-pH} + A_A\, 10^{-pKa}}{10^{-pH} + 10^{-pKa}} \qquad \text{(S1)}$$

Fluorescence spectroscopy at different temperatures. Solutions of the acridizinium derivative **2** in anhydrous glycerol ($c = 1.0 \times 10^{-5}$ M) were recorded in the range 0–100 °C in 5–10 °C steps, using a thermoelectric temperature controller. Between temperature changes, the samples were left for 10–15 min to achieve thermal equilibration. The changes in the molar concentration of the acridizinium derivative, caused by the thermal expansion of the solvent, were not taken into account.

***N*-(2′-aminobiphenyl-2-yl)acridizinium-9-carboxamide perchlorate** (**2**). 9-Carboxyacridizinium bromide (780 mg, 2.56 mmol) was mixed with polyphosphoric acid (12.0 g) and stirred under argon gas atmosphere at 120 °C until HBr evolution ceased and a homogeneous mixture formed (ca. 30 min). Then, 2,2′-diaminobiphenyl (472 mg, 2.56 mmol) was added, and the reaction mixture was vigorously stirred under argon-gas atmosphere at 160 °C for 5 h. After cooling to 100 °C, water (30 mL) was added, whereas a yellow precipitate separated. The reaction mixture was stirred at this temperature for additional 10 min, then cooled to room temperature, diluted with 70 g of ice and, after stirring for 30 min, the turbid solution was filtered. To the filtrate, saturated aqueous solution of $NaClO_4$ (10 mL) was added. The suspension was extracted with nitromethane (4 × 100 mL); the combined organic layers were washed with water, and the solvent was removed in vacuo, to give **2** (2.19 g, 4.59 mmol, 47%) as yellow ^{1}H-NMR-spectroscopically pure solid. An analytically pure sample was obtained by recrystallization from MeCN–water (1:1 v/v) as dark-yellow needles, m.p. 240–242 °C (dec.). ^{1}H-NMR (DMSO-d_6, 400 MHz): δ = 4.98 (s, 2H, NH_2), 6.73 (ddd, 1H, ^3J = 7.5 Hz, ^3J = 7.5 Hz, ^4J = 1.0 Hz, 5-H''), 6.90 (d, 1H, ^3J = 7.8 Hz, 3-H''), 7.07 (dd, 1H, ^3J = 7.7 Hz, ^4J = 1.0 Hz, 6-H''), 7.11 (ddd, 1H, ^3J = 7.5 Hz, ^3J = 7.5 Hz, ^4J = 1.5 Hz, 4-H''), 7.40 (ddd, 1H, ^3J = 7.6 Hz, ^3J = 7.6 Hz, ^4J = 1.1 Hz, 5-H'), 7.42 (m, 1H, 6-H'), 7.49 (ddd, 1H, ^3J = 8.1 Hz, ^3J = 6.2 Hz, ^4J = 2.0 Hz, 4-H'), 7.80 (d, 1H, ^3J = 7,9 Hz, 3-H'), 8.03 (ddd, 1H, ^3J = 7.0 Hz, ^3J = 7.0 Hz, ^4J = 1.2 Hz, 3-H), 8.15 (m, 1H, 2-H), 8.17 (m, 1H, 7-H), 8.53 (d, 1H, ^3J = 8.9 Hz, 1-H), 8.64 (d, 1H, ^3J = 8.9 Hz, 8-H), 8.66 (s, 1H, 10-H), 9.31 (s, 1H, 11-H), 9.33 (d, 1H, ^3J = 7.2 Hz, 4-H), 10.42 (s, 1H, NH), 10.47 (s, 1H, 6-H). – ^{13}C-NMR (DMSO-d_6, 100 MHz): δ = 116.0 (CH, 3-C''), 117.6 (CH, 5-C''), 123.1 (CH, 3-C), 124.4 (C_q), 126.1 (CH, 3-C'), 126.2 (CH, 8-C), 126.3 (CH, 6-C'), 126.5 (CH, 11-C), 126.7 (CH, 10-C), 127.1 (C_q), 127.8 (CH, 4-C'), 128.5 (CH, 2-C), 128.6 (CH, 4-C'), 128.7 (CH, 1-C),

[4] Polster, J.; Lachmann, H. *Spectrometric Titrations: Analysis of Chemical Equilibria*, VCH, Weinheim, **1989**.

130.7 (CH, 7-C), 130.8 (CH, 6-C''), 131.0 (CH, 5-C'), 131.8 (C_q), 133.8 (CH, 4-C), 134.4 (C_q), 134.6 (C_q), 135.0 (C_q), 138.0 (C_q), 140.3 (CH, 6-C), 144.8 (C_q), 163.9 (C_q). – IR: $\tilde{\nu}$ [cm^{-1}] = 3406 (NH_2), 1672 (-CONHR). – MS (ESI^+); *m/z* (%) = 391 (27) $[M+H]^+$, 390 (100) $[M]^+$. – El. Anal. for $C_{26}H_{20}ClN_3O_5$ (489.9 g/mol): calc. (%) C 63.7, H 4.11, N 8.58; found (%) C 63.9, H 4.10, N 8.67.

9-(Benzimidazol-2-yl)acridizinium bromide (3). 9-Carboxyacridizinium bromide (912 mg, 3.00 mmol) was mixed with polyphosphoric acid (12.0 g) and heated under argon-gas atmosphere at 120 °C until HBr evolution ceased and a homogeneous mixture formed (ca. 30 min). Then, *o*-phenylenediamine (324 mg, 3.00 mmol) was added, and the reaction mixture was vigorously stirred under argon-gas atmosphere at 160 °C for 4 h. After cooling to 100 °C, water (100 mL) was added (*CAUTION! HEAT SURGE POSSIBLE*), and a yellow solid precipitated. The reaction mixture was stirred at 160 °C for 30 min, then cooled to room temperature, diluted with 20 g of ice and transferred into a large beaker. The mixture was neutralized by slow addition of 20% aq. Na_2CO_3 (*faster addition results in red coloration and decomposition of the product*) until the yellow solid dissolved (final pH ca. 6). The solution was filtered; the residue on the filter was washed with ca. 10 mL water, and the filtrate was treated with a solution of $NaClO_4 \times H_2O$ (4.2 g, 30 mmol) in ca. 10 mL water, resulting in immediate precipitation of a voluminous yellow solid. After standing at 4 °C overnight, the precipitate was separated, washed with cold water (3 × 10 mL) and acetone (3 ×20 mL). After drying *in vacuo*, the yellow perchlorate was suspended in MeCN (20 mL) and the solution was stirred under reflux. Dry DMF (ca. 40 mL) was added to the refluxing solution until the solid dissolved almost completely. The hot solution was filtered, and *n*-Bu_4NBr (9.7 g, 30 mmol) in MeCN (10 mL) was added, whereas yellow precipitate has separated. After standing at 4 °C overnight, the precipitate was collected, washed with MeCN (3 × 10 mL), AcOEt (2 × 10 mL), to give compound **3** (0.64 g, 57%) as yellow amorphous powder. Recrystallization from boiling 50% aq. EtOH gave 0.66 g of the monohydrate as bright-yellow fine needles, which loose water upon drying at 120 °C (0.02 mbar) with a color change to orange; m.p. > 340 °C. ^{1}H NMR (400 MHz, DMSO-d_6 – CD_3OD): δ = 7.26 (br s, 2 H, 2 Ar-H), 7.60 (br s, 1 H, Ar-H), 7.68 (br s, 1 H, Ar-H), 7.93 (dd, ^3J = 6.8 Hz, 1 H, H-3), 8.08 (dd, ^3J = 8.2 Hz 1 H, H-2), 8.54–8.59 (m, 2 H, H-1, H-8), 8.69 (d, ^3J = 8.9 Hz, 1 H, H-7) 8.95 (s, 1 H, H-11), 9.16 (s, 1 H, H-10), 9.24 (d, ^3J = 6.9 Hz, 1 H, H-4), 10.30 (s, 1 H, H-6), 13.36 (br s, 1 H, NH). – ^{13}C-NMR (DMSO-d_6 – CD_3OD, 100 MHz): 122.7 (CH), 123.6 (CH), 124.0 (CH), 125.3 (CH), 126.0 (CH), 127.1 (CH), 128.7 (CH), 129.0 (CH), 131.6 (CH), 134.5 (CH), 134.8 (C_q), 135.6 (C_q), 138.3 (C_q), 139.8 (CH), 149.0 (C_q) (two C_q not detected). – MS (ESI) *m/z* (%) = 296 (100) $[M]^+$. – $C_{20}H_{14}BrN_3$ (FW 376.25): calcd. C 63.84, H 3.75, N 11.17; found C 64.05, H 3.74, N 11.18.

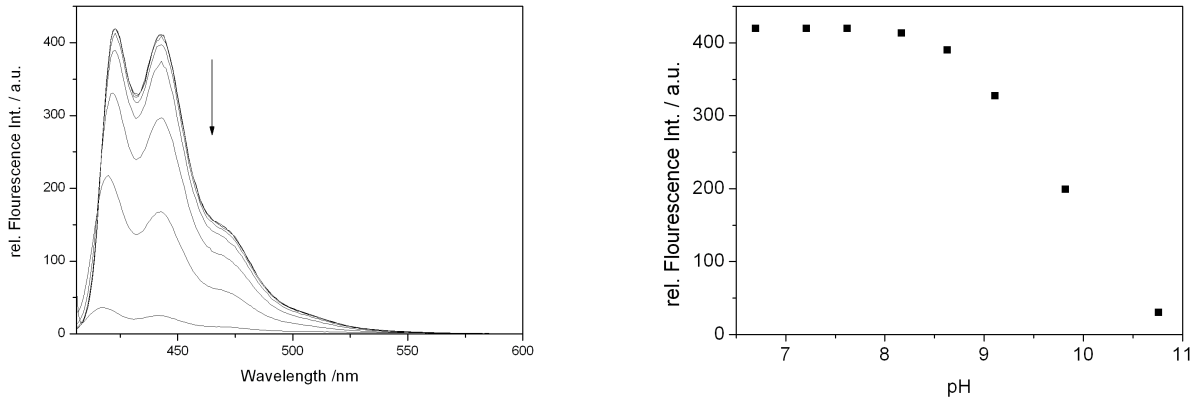

Figure S1. Left: Spectrofluorimetric titration of base to acridizinium derivative **1a** (c = 1.8 × 10^{-4} M in Britton-Robinson buffer); arrows indicate the development of emission bands upon addition of base; right: plot of the relative emission intensity of **1a** *vs* pH of the solution.

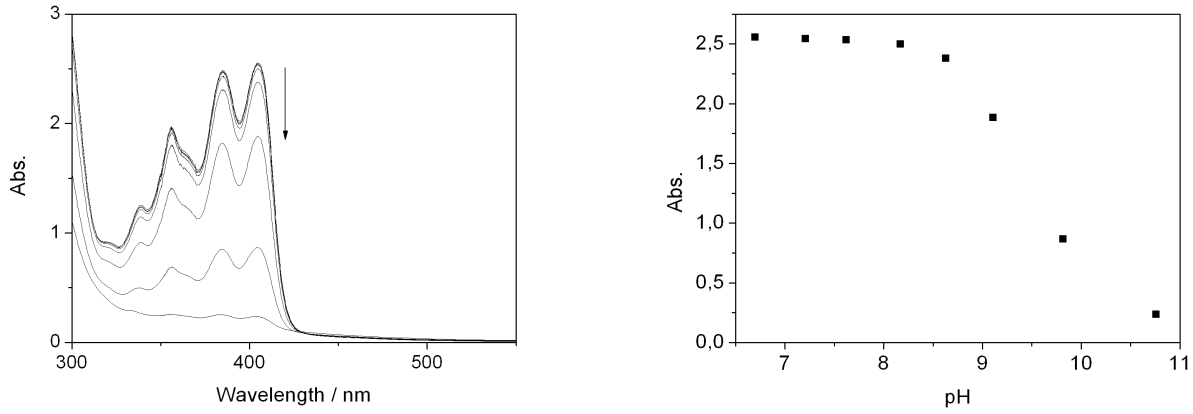

Figure S2. Left: Spectrophotometric titration of base to acridizinium derivative **1a** (c = 1.8 × 10^{-4} M in Britton-Robinson buffer); arrows indicate the development of absorption bands upon addition of base, right: plot of the absorbtion of **1a** *vs* pH of the solution.

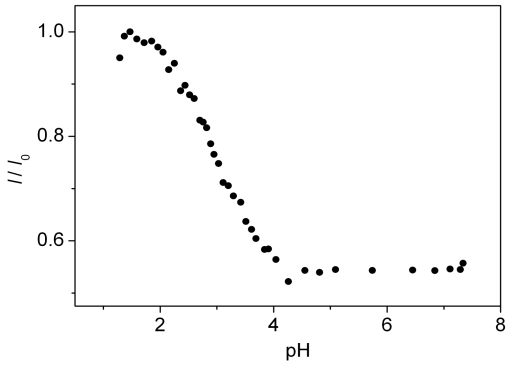

Figure S3. Dependence of the pH value on the emission intensity of acridizinium derivative **2** (c = 1.0 × 10^{-5} M in Britton-Robinson buffer) presented as plot of the relative fluorescence intensity *vs* the pH of the solution; λ_{ex} = 300 nm.

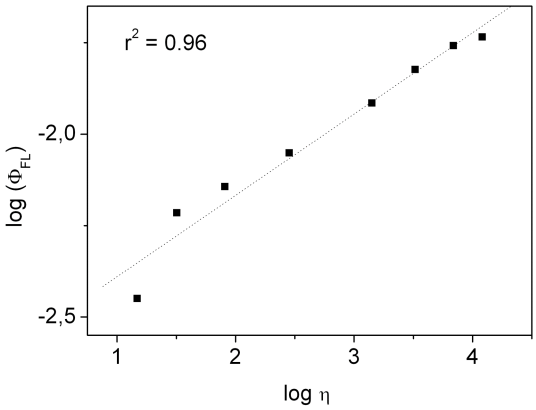

Figure S4. Dependence of the fluorescence quantum yield, ϕ_{Fl}, of the acridizinium derivative **2** on the viscosity, η, of the glycerol solution given as plot of log ϕ_{Fl} versus log η; different viscosities of the glycerol solution were adjusted by variation of the temperature.

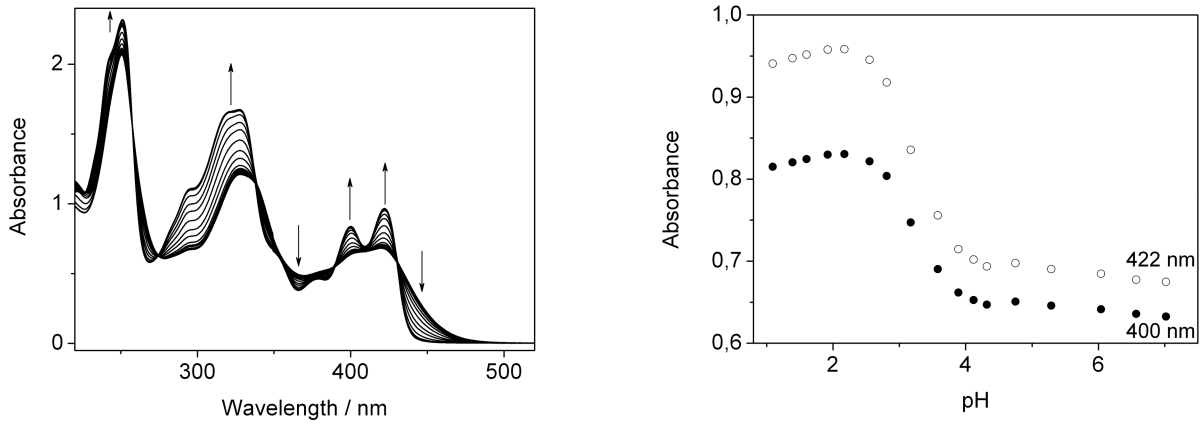

Figure S5. Left: Spectrophotometric titration of acid to acridizinium derivative **3** ($c = 1.0 \times 10^{-4}$ M in Britton-Robinson buffer); arrows indicate the development of absorption bands upon addition of acid (from pH = 7.0 to pH = 1.0); right: plot of the absorption of **3** at 400 and 422 nm *vs* the pH of the solution (the decrease of absorption at pH < 2 is likely due to partial hydrolysis of the benzimidazole substituent under these conditions).