Electronic Supplementary Information

A molecular probe for the optical detection of biogenic amines

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General: Sodium dodecyl sulfate (SDS), coumarin 466, putrescine, cadaverine, histamine, 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES), 3-*N*,*N*-dimethylaminophenol, *n*-propylamine, L-cysteine (Sigma-Aldrich), 3-(N-morpholino)propanesulfonic acid (MOPS), ethanolamine, glycine, L-histidine, spermidine, spermine, malonic acid, phosphoryl chloride, glutamic acid (Fluka), tyramine, *n*-butylamine, γ-aminobutyric acid (GABA) (Acros) and tryptamine (Applichem GmbH) were purchased and used as received. HEPES buffer (100 mM, pH 7.4) was prepared with bidistilled water and used for all experiments. The synthesis of probe **1** is described in *Org. Lett.*, 2011, **13**, 1658.[1] Stock solutions of **1** (0.185 mM) + SDS (120 mM) and of the amines (10 mM) were prepared in bidistilled water and stored at 4°C. All UV-Vis spectra were recorded on a Lambda 35 spectrometer (Perkin Elmer). Fluorescence measurements were recorded on a Varian Cary Eclipse spectrophotometer equipped with a thermostatted cell holder. High resolution mass spectra were recorded with a waters Q - TOF Ultima (ESI-TOF) instrument.

Sensing experiments: Solutions containing coumarin **1**, SDS, HEPES buffer and the amine analyte were prepared by mixing appropriate amounts of the stock solutions in 3.0 ml vials. The vials were placed on an aluminum heating plate and heated at 50 °C for 2 h. Subsequently, the solutions were transferred into cuvettes and UV-Vis spectra and fluorescence emission spectra (λ_{ex} = 377 nm) were recorded. Each series of measurements was repeated three times. The final concentrations were: [dye] = 9.3 μM, [SDS] = 6.0 mM, [amine] = 0.50 mM, and [HEPES] = 50 mM.

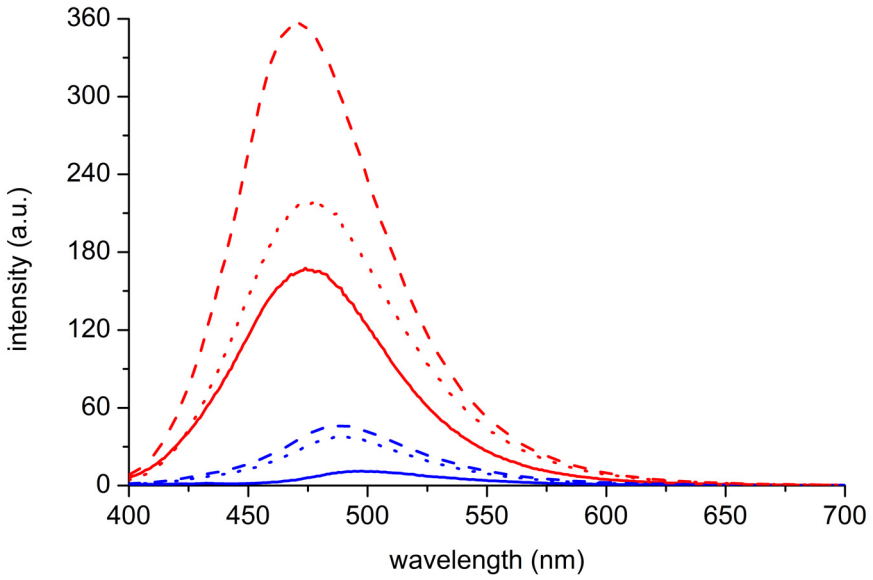


Fig. S1 Fluorescence emission spectra (λ_{ex} = 377 nm) of buffered aqueous solutions (50 mM HEPES, pH 7.4) containing molecular probe **1** (9.3 μM), SDS (6.0 mM) and 0.50 mM of histidine (hashed blue line), cysteine (dotted blue line), histamine (solid red line), spermine (hashed red line), cadaverine (dotted red line), or no amine (solid blue line). Prior to the measurement, the solutions were tempered for 2 h at 50 °C.

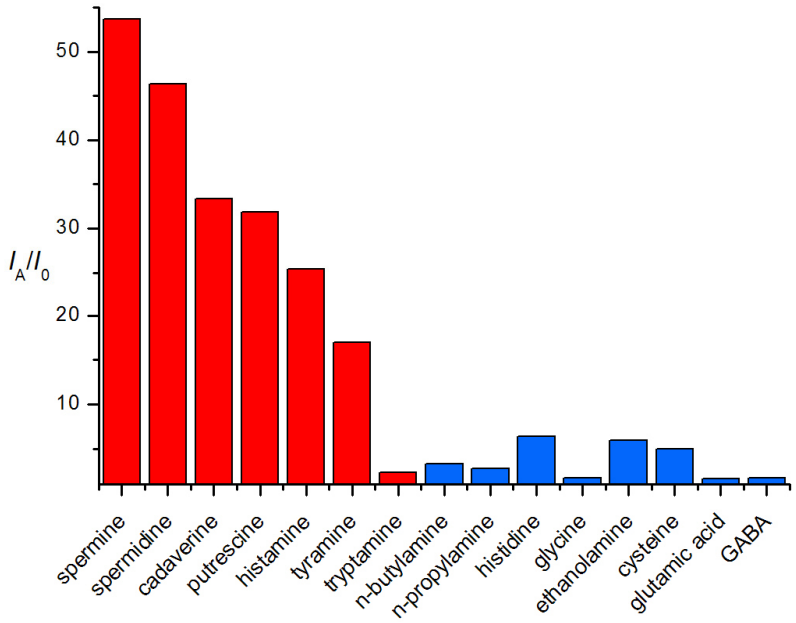


Fig. S2 Changes of fluorescence emission intensity at 472 nm (λ_{ex} = 377 nm) of buffered aqueous solutions (50 mM HEPES, pH 7.4) containing molecular probe **1** (9.3 μM) and SDS (6.0 mM) after addition of different amines (0.50 mM). Prior to the measurement, the solutions were tempered for 2 h at 50 °C. Important biogenic amines are shown in red and other amines in blue.

Spectrophotometric titrations: Stock solutions of histamine (10 mM), *n*-propylamine (10 mM), dye (0.185 mM) + SDS (120 mM), and HEPES buffer (100 mM, pH 7.4) were used to prepare a series of solutions with constant concentrations of dye **1** (9.3 μM), SDS (6.0 mM) and HEPES buffer (50 mM), and a variable concentration of the amine (0–1.2 mM) in 3.0 ml vials. The vials were placed on an aluminum heating plate and heated at 50 °C for 2 h. Subsequently, the solutions were transferred into cuvettes and UV-Vis spectra were recorded in the range 300550 nm.

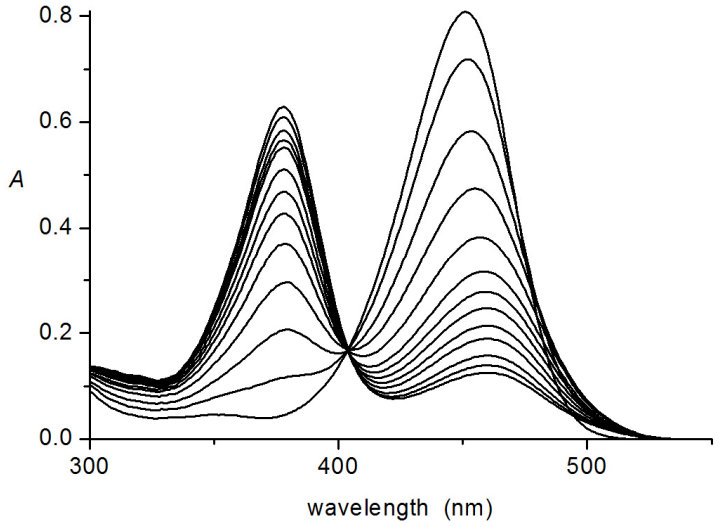
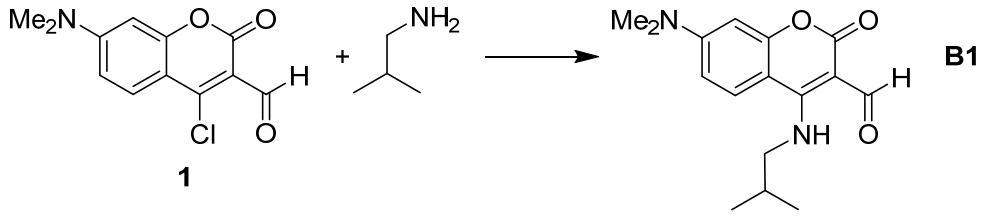


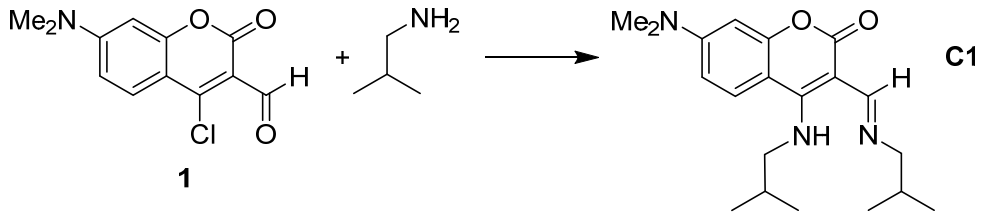
Fig. S3 Absorption spectra of buffered aqueous solutions (50 mM HEPES, pH 7.4) containing molecular probe **1** (9.3 μM), SDS (6.0 mM) and different amounts of histamine (0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2 mM). Prior to the measurement, the solutions were tempered for 2 h at 50 °C. Increasing histamine concentration lead to a reduced absorption at 451 nm and an increase at 377 nm with an isosbestic point at 403 nm.

Synthesis of B1:



Coumarin **1** (35 mg, 0.14 mmol) was combined with *i*-butylamine (38 µL, 0.38 mmol) in a mixture of CH_3OH and $CHCl_3$ (7:3, 1.0 mL). The solution was stirred at RT for 15 min and the solvents were removed under vacuum. The crude product was crystallized from ethanol. To remove traces of *i*-butylamine, the resulting material was suspended in water and the solvent was removed under vacuum at 70 °C and dried for 30 min. This procedure was repeated twice to give enamine **B1** (24 mg, 82 µmol) in 67 % yield. ^{1}H-NMR ($CD_3OD/CDCl_3$, 7:3, 400 MHz) δ 10.00 (s, 1 H, CHO), 8.00 (d, J = 10 Hz, 1 H, CH_{arom}), 6.74 (dd, J = 3 and 10 Hz, 1 H, CH_{arom}), 6.53 (d, J = 3 Hz, 1 H, CH_{arom}), 3.73 (d, J = 7 Hz, 2 H, CH_2), 3.18 (s, 6 H, NCH_3), 2.10 (m, 1 H, CH), 1.20 (d, J = 7 Hz, 6 H, CH_3). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 191.00, 164.08, 159.55, 157.66, 154.03, 129.12, 108.31, 102.14, 98.71, 94.84, 55.26, 39.93, 29.21, 20.32. *m/z* (high resol. TOF-MS ES$^+$) 289.1552 (calc. for $C_{16}H_{20}N_2O_3$ x H^+: 289.1552).

Synthesis of C1:



Coumarin **1** (20 mg, 79.5 µmol) was combined with *i*-butylamine (34 µL, 0.34 mmol) in a mixture of CH_3OH and $CHCl_3$ (7:3, 1.0 mL). The solution was stirred at RT for 15 min and solvents were removed under vacuum. The crude product was crystallized from hexanes to give pale yellow crystals of **C1** (11 mg, 32 µmol) in 55% yield. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 12.58 (s, br, 1 H, NH), 8.67 (s, 1 H, N=CH), 7.78 (d, J = 10 Hz, 1 H, CH_{arom}), 6.47 (dd, J = 3 and 10 Hz, 1 H, CH_{arom}), 6.42 (d, J = 3 Hz, 1 H, CH_{arom}), 3.53 (d, J = 7 Hz, 2 H, NCH_2), 3.26 (d, J = 7 Hz, 2 H, NCH_2), 2.98 (s, 6 H, NCH_3), 1.95 (s, J = 7 Hz, 1 H, $CH(CH_3)_2$), 1.81 (s, J = 7 Hz, 1 H, $CH(CH_3)_2$), 1.01 (d, J = 7 Hz, 6 H, $CH(CH_3)_2$), 0.87 (d, J = 7 Hz, 6 H, $CH(CH_3)_2$). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 164.49, 162.06, 157.72, 156.86, 153.16, 128.30, 108.13, 104.40, 99.33, 90.62, 69.49, 55.97, 40.34, 30.55, 29.98, 27.38, 21.12, 20.89. *m/z* (high resol. TOF-MS ES$^+$) 344.2328 (calc. for $C_{20}H_{29}N_3O_2$ x H^+: 344.2338).

NMR spectroscopic analysis:

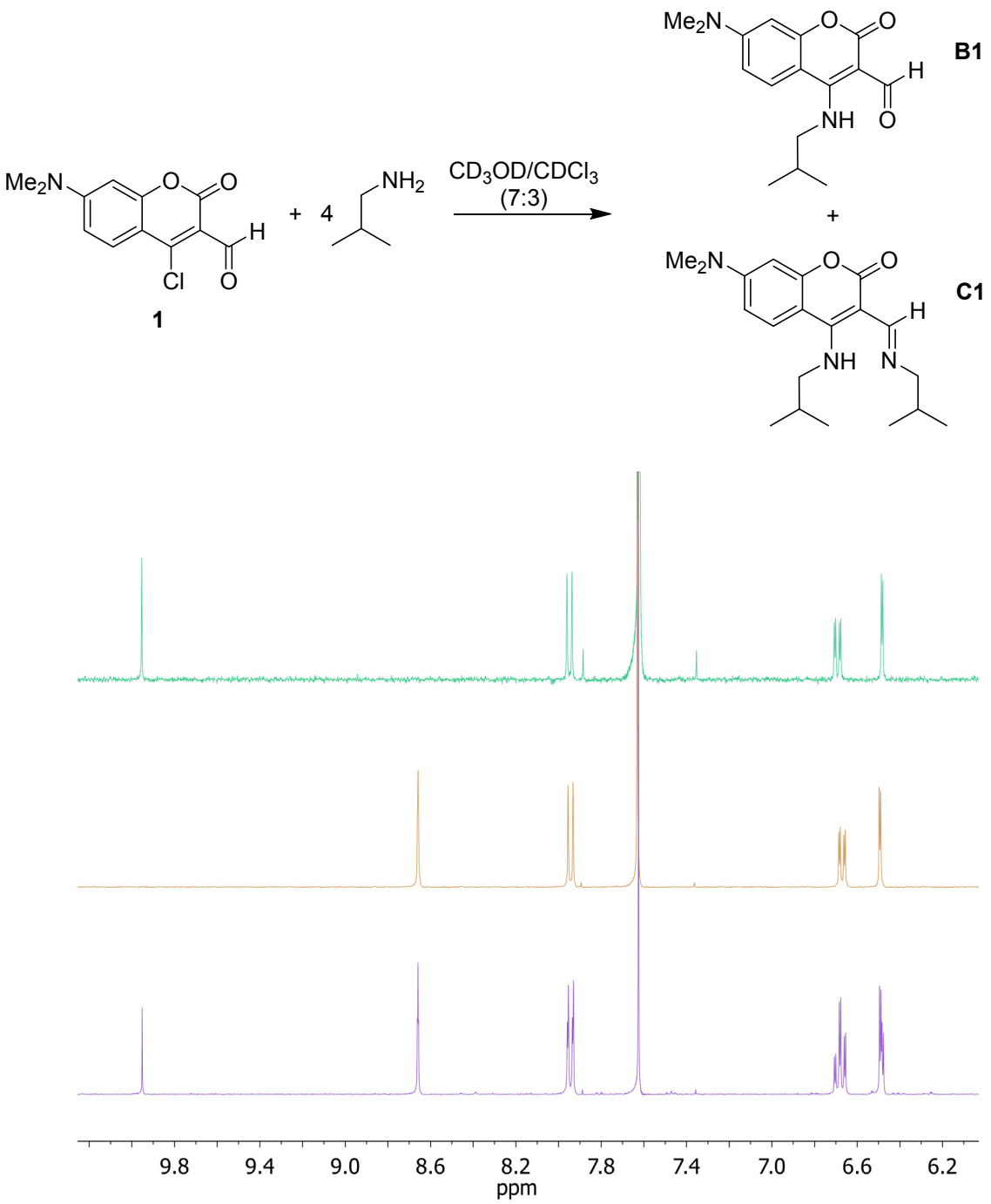


Fig. S4 Part of the ^{1}H NMR ($CD_3OD/CDCl_3$, 7:3) spectra of coumarin **B1** (top), coumarin **C1** (middle), and of a mixture of coumarin **1** (4.0 mM) and *i*-butylamine (16.8 mM) after an incubation time of 10 min.

Crystallographic analysis of C1: Single crystals of **C1** were obtained from hexanes. Intensity data were collected using an Oxford Diffraction KM-4 CCD diffractometer having kappa geometry and using graphite monochromatized Mo-K_α radiation (λ = 0.71073 Å) at low temperature. Data reduction was carried out with *automar*.[2] Structure solution and refinement were performed with the SHELXTL software package.[3] The structures were refined using the full-matrix least-squares routines on F^2. All non-hydrogen atoms were refined with anisotropic displacement parameters. H atoms were included to the models in calculated positions using the riding model. More detailed information can be found in the corresponding CIF file.

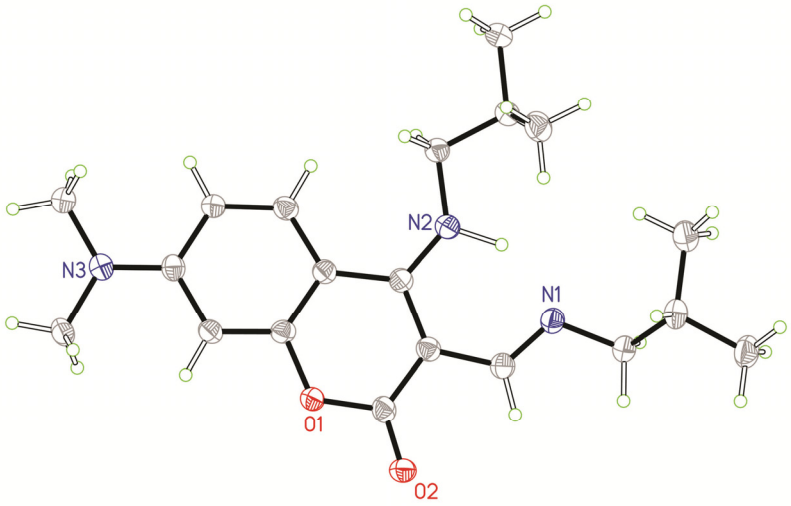
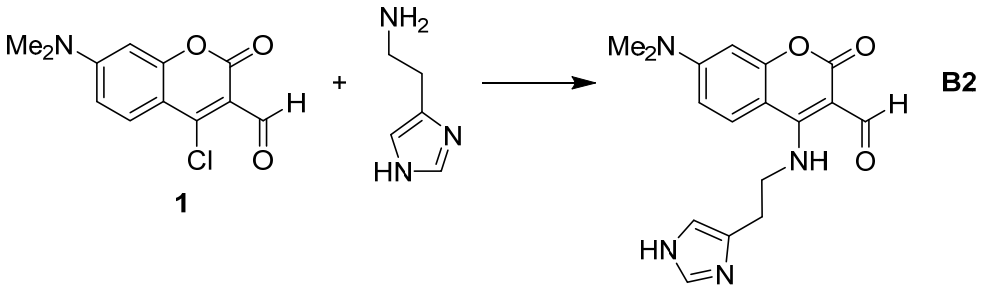


Fig. S5 ORTEP representation of the molecular structure of coumarin **C1** in the solid state.

Synthesis of B2:



Coumarin **1** (15 mg, 59.6 µmol) was combined with histamine (18 mg, 0.16 mmol) in a mixture of CH_3OH and $CHCl_3$ (7:3, 1.0 mL). The solution was stirred at RT for 10 min and solvents were removed under vacuum. The crude product was dissolved in chloroform (2.0 ml) and washed with water (8.0 ml, 5 times) to give **B2** (4.2 mg, 13 µmol) in 28% yield. ^{1}H-NMR ($CD_3OD/CDCl_3$, 7:3, 400 MHz) δ 9.89 (s, 1 H, CHO), 8.11 (s, 1 H, $CH_{imidazole}$), 7.99 (d, J = 10 Hz, 1 H, CH_{arom}), 7.23 (s, 1 H, $CH_{imidazole}$), 6.71 (dd, J = 4 and 10 Hz, 1 H, CH_{arom}), 6.48 (d, J = 4 Hz, 1 H, CH_{arom}), 4.18 (t, J = 7 Hz, 2 H, CH_2), 3.16 (t, J = 7 Hz, 2 H, NCH_2),

3.14 (s, 6 H, NCH_3). ^{13}C-NMR (MeOD, 100 MHz) δ 191.51, 166.38, 160.89, 158.89, 156.27, 136.19, 130.75, 118.61, 110.40, 102.56, 99.43, 95.83, 63.45, 40.23, 30.94, 26.97. *m/z* (high resol. TOF-MS ES^+) 327.1734 (calc. for $C_{19}H_{19}N_4O_3$ x H^+: 327.1457).

UV-Vis analysis of B2:

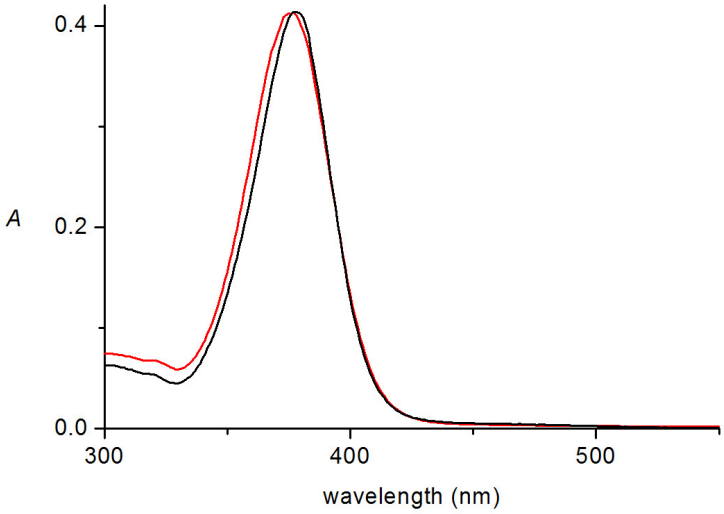


Fig. S6 UV-Vis spectrum of buffered aqueous solutions (50 mM HEPES, pH 7.4) containing SDS (6.0 mM) and coumarin **B2** (red line) or a mixture of **1** (9.3 μM) and histamine (0.5 mM) after an incubation time of 36 h. The spectrum of **B2** was normalized so that the maximum intensity at 377 nm matches the one found for the mixture of **1** and histamine.

Calibration curves for quantitative measurements:

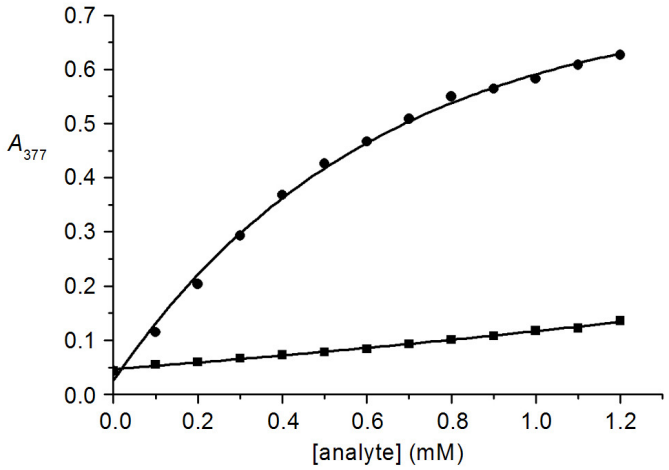


Fig. S7 Absorption at 377 nm of buffered aqueous solutions (50 mM HEPES, pH 7.4) containing molecular probe **1** (9.3 μM), SDS (6.0 mM) and different amounts of histamine (spheres) or *n*-propylamine (squares). Prior to the measurement, the solutions were tempered for 2 h at 50 °C. The values represent averages of two independent measurements. The curve could be used for quantitative histamine measurements in the range of ~ 0.1 – 1.0 mM. The selectivity for histamine over *n*-propylamine is good; the initial rates of the reaction (derived from the absorption at low analyte concentrations) differ by a factor of 14.

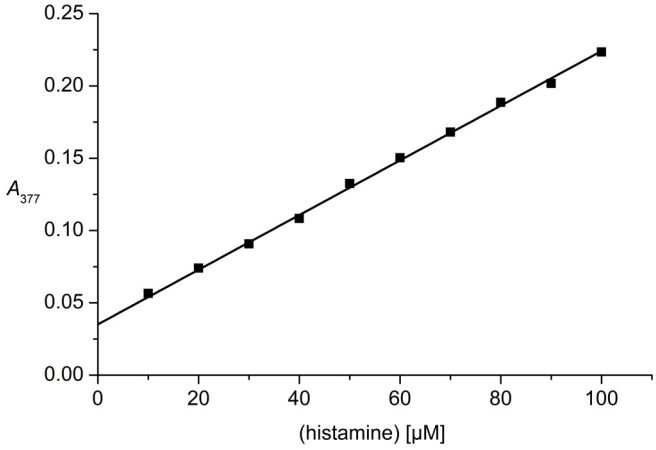


Fig. S8 Absorption at 377 nm of buffered aqueous solutions (50 mM HEPES, pH 7.4) containing molecular probe **1** (9.3 μM), SDS (6.0 mM) and different amounts of histamine. Prior to the measurement, the solutions were tempered for 6 h at 50 °C. The curve could be used for quantitative histamine measurements in the range of ~ 10 – 100 μM.

Effect of the pH

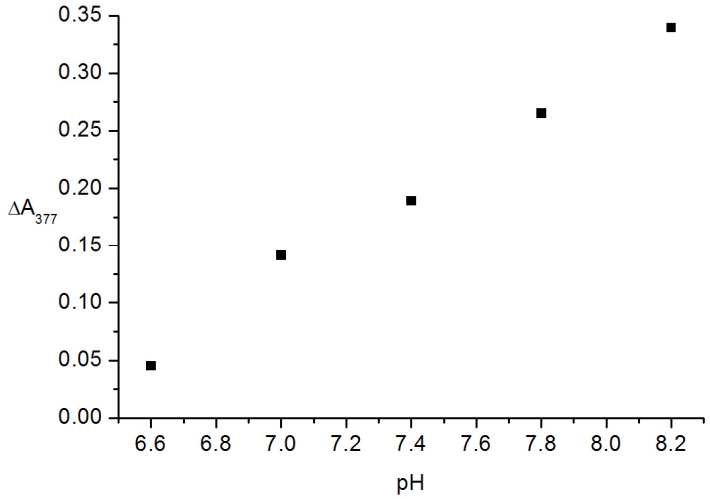


Fig. S9 Difference of the absorption at 377 nm for solutions containing probe **1** (9.3 µM) and SDS (6.0 mM) with and without histamine (0.30 mM) as a function of pH. MOPS buffer was used for pH = 6.6 and 7.0, and HEPES buffer was used for pH = 7.4, 7.8, and 8.2. Prior to the measurement, the solutions were tempered for 2 h at 50 ˚C.

Sensing of amines in the gas phase:

Preparation of slides: A mixture of poly(methyl methacrylate) (PMMA, av. molecular weight ca. 350'000, Sigma-Aldrich) (95 mg, 0.095 µmol) and coumarin **1** (0.5 mg, 2.0 µmol) was dissolved in chloroform (1.0 mL). 250 µL of the solution were drop coated on a 2.5 x 2.5 cm^2 glass slide. The slides were transferred into a desiccator and dried over night at RT.

Sensing experiment: 500 µL of the biogenic amine putrescine, the primary amine *n*-butylamine, the secondary amine diethylamine, and ammonia (aqueous solution, 25%) were added to 24.0 mL glass vials. The slides were placed on top of vials with the polymer layer facing the inside of the vials. After 2 h at RT, the originally yellow color of the polymer layer has faded away in the case of the biogenic amine putrescine and the primary amine *n*-butylamine. Minor changes of color were observed for diethylamine and ammonia (Fig. S10).

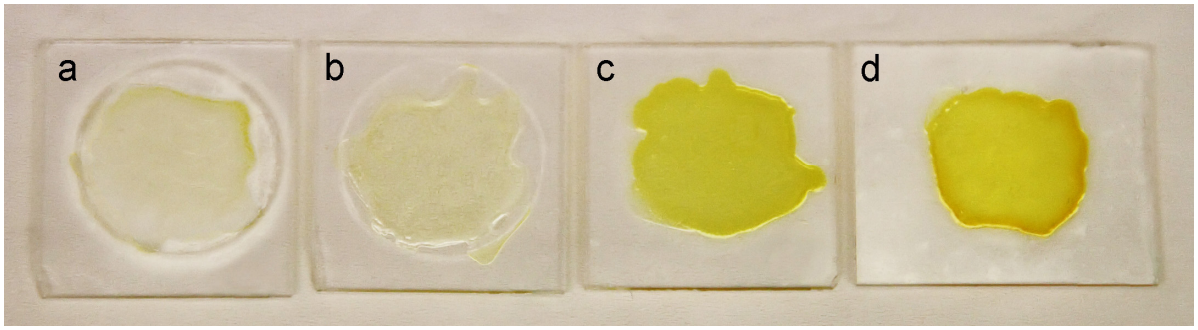


Fig. S10 Photographs of glass slides with a drop-coated PMMA layer containing coumarin **1**. The slides were exposed for 2 h to vapors of putrescine (a), *n*-butylamine (b), diethylamine (c), and ammonia (d).

References:

[1] J.-J. Chen, K.-T. Li and D.-Y. Yang, *Org. Lett.*, 2011, **13**, 1658.
[2] *Automar*, release 2.7, Claudio Klein, Marresearch GmbH, Germany, 2010.
[3] G. M. Sheldrick, University of Göttingen, Germany, 1997; *SHELXTL*, release 6.1.4, Bruker AXS Inc., Madison, Wisconsin 53719, USA, 2003.