Supporting Information

Light-Switchable Buffers

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anie_202109250_sm_misellaneous_information.pdf
Supplementary Information

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**1. General Information**

All reagents and solvents were purchased from commercially suppliers (Sigma-Aldrich, TCI, Acros Organics, Fluorochem, Abcr) and used without further purification. Compound II3 was synthesized and purified as described previously. H₂O used in all preparations was filtered with a MilliQ-Integral5 purification system (mQ H₂O).

NMR spectra were recorded at 298 K on the following spectrometers: Bruker Avance III-400 equipped with a BBFO₂ probe, Bruker Avance 400 equipped with a BBI₂ probe, Bruker Avance NEO-400 equipped with a BBFO probe (characterization), and on a Bruker Avance III HD-600 equipped with a CPPBO₂ ATMA probe (pH titrations). Chemical shifts (δ) are reported in parts per million (ppm), while coupling constants (J) are given in Hertz (Hz). Solvent residual signals are used as internal reference for spectra alignment (CDCl₃: δ = 7.26 ppm, DMSO-d₆: δ = 2.50 ppm, D₂O: δ = 4.87 ppm).

Electrospray-ionization HRMS analyses were run (positive mode/direct injection) on a Waters XEVO G2-S GTOF spectrometer interfaced with Acquity UPLC pumps and sample manager system.

UV-Vis spectra and kinetics were acquired on an Agilent Cary 60 spectrometer equipped with an 18-cell holder coupled to a Huber thermostat, using Suprasil quartz cuvettes (114-QS) from Hellma Analytics.

pH measurements were performed using Metrohm pH module 867 or Titrando 888 coupled with Unitrode Pt1000 or Biotrode glass electrodes; pH data were processed using the software Metrohm Tiamo Light.

Samples photoirradiation was carried out using an Agilent Cary 60 spectrometer equipped with a Prizmatix FC-LED-425Z or FC-LED-500Z high-power LED light sources (λ = 425 or 500 nm, respectively). The light beam was delivered by polymer optical fibers (core 1500 μM) i) positioned orthogonally to and just below the liquid/air interface of sample solutions (UV-Vis) or ii) connected to an FCM1-06 collimator coupled with a 45° mirror cage, resulting in a 25 mm light beam tilted by 90° (pH jumps). Power measurements of the fiber-coupled LED output were made with Thorlabs S142C integrating sphere photodiode power sensor; the uncertainty is within 3-5%.

Potassium phosphate buffers from pH 3 to 10 were prepared titrating a solution of H₃PO₄ (100 mM, 1L) with KOH 10 M. Diluted stocks of H₃PO₄ or HCl were used below pH 3. All buffer stocks were stored at room temperature. In order to account for the pH change in sample preparations, the pH of each buffer stock is measured again after dilution (1/5, 20 mM) in mQ H₂O.

Unless stated otherwise, UV-Vis, ¹H NMR and pH jump analyses were carried out and analysed as described previously. In the case of UV-Vis titrations the error on pH values results from multiple reading before and after the experiments (≤ ± 0.04), whereas in the case of NMR samples we account for an uncertainty of ± 0.1 pH units.
2. Synthetic Procedures

![Synthetic route for the synthesis of 1-4; o/p- refers to the ene-iminium core.](image)

Scheme S1. Synthetic route for the synthesis of 1-4; o/p- refers to the ene-iminium core.

### 2.1 Synthesis of 2,3,3-trimethylindolines, II–2

**II1**: In a 250-ml round bottom flask, p-methoxyphenylhydrazine hydrochloride (5.12 g, 29.3 mmol) and isopropyl methyl ketone (3.8 ml, 35.0 mmol) were dissolved in glacial AcOH (85 mL) and the mixture was heated to reflux under N₂ atmosphere for 24 h. After evaporation of the solvent under reduced pressure, the residues were dissolved in DCM and the resulting organic layer washed with brine (3 × 100 mL), satd. Na₂CO₃ (3 × 100 mL) and dried over anhydrous MgSO₄. Solvent was removed under reduced pressure and the crude product purified by flash chromatography (SiO₂, hexane/EtOAc 2:1). Fractions containing the product were kept dry by rotary evaporation. The obtained yellow oily residue was further purified by sublimation under vacuum, yielding II1 as a white crystalline solid (3.49 g, 62.8 %). ¹H NMR (400 MHz, DMSO-d₆) δ (ppm): 7.30 (d, J = 8.4 Hz, 1H), 7.03 (d, J = 2.5 Hz, 1H), 6.80 (dd, J = 8.4, 2.5 Hz, 1H), 3.76 (s, 3H), 2.15 (s, 3H), 1.22 (s, 6H). ¹³C NMR (101 MHz, DMSO-d₆) δ (ppm): 185.22, 157.37, 147.52, 147.11, 119.45, 112.16, 108.15, 55.41, 53.29, 22.63, 14.88.

**II2**: The same procedure above is applied. It was noted, however, that compound II2 is rather unstable as compared to II1. Thus, the oily residue obtained after flash chromatography was used in the next step without further purification. ¹H NMR (400 MHz, CDCl₃) δ (ppm): 7.10 (t, J = 7.8 Hz, 1H), 6.83 (d, J = 7.8 Hz, 1H), 6.79 (d, J = 7.8 Hz, 1H), 3.90 (s, 3H), 2.22 (s, 3H), 1.22 (s, 6H). ¹³C NMR (101 MHz, CDCl₃) δ (ppm): 186.36, 150.91, 147.67, 141.48, 126.24, 113.58, 110.05, 55.59, 53.98, 22.95, 15.27. HRMS (ESI/QTOF) m/z: [M + H]⁺ Calcd for C₁₂H₁₆NO⁺ 190.1226; Found 190.1228.

### 2.1 Synthesis of 2,3,3-trimethylindolium-propyl-1-sulfonates, III–2

**III1**: In a 100-mL round bottom flask III1 (1.10 g, 5.8 mmol) and 1,3-propanesultone (1.21 g, 9.9 mmol) of were dissolved in MeCN (20 mL) and the solution heated to reflux for 16 h. The resulting mixture
was cooled down to room temperature and added dropwise to EtOAc under stirring, leading to a white-green precipitate. The solid was separated by vacuum filtration, washed with EtOAc (3 × 10 mL) and recrystallized from hot MeCN, yielding **II1** as a purple solid (1.07 g, 59.1%). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ (ppm): 7.95 (d, $J = 8.8$ Hz, 1H), 7.45 (d, $J = 2.5$ Hz, 1H), 7.15 (dd, $J = 8.8, 2.5$ Hz, 1H), 4.61 (t, $J = 7.5$ Hz, 2H), 3.86 (s, 3H), 2.76 (s, 3H), 2.60 (t, $J = 6.6$ Hz, 2H), 2.13 (p, $J = 7.1$ Hz, 3H), 1.51 (s, 6H). $^{13}$C NMR (101 MHz, DMSO) $\delta$ ppm: 193.54, 160.56, 144.00, 134.38, 116.42, 114.30, 109.37, 56.05, 53.91, 47.33, 46.54, 23.86, 22.12, 13.50. HRMS (ESI/QTOF) m/z: [M + H]$^+$ Calcd for C$_{15}$H$_{22}$NO$_4$S $^+ 312.1264$; Found 312.1266.

**II2:** In a 50-mL round bottom flask I$_2$ (0.80 g, 4.2 mmol) and 1,3-propanesultone (0.52 g, 4.2 mmol) were added and the flask sealed with a septum under N$_2$ atmosphere. The mixture was heated up at 120 °C under stirring for 15 minutes. The dark brown mixture was treated with MeCN (20 mL) and the resulting precipitate filtered, washed with MeCN (3 × 20 mL) and dried, yielding **II2** a beige solid (0.27 g, 20%). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ (ppm) 7.57 (t, $J = 7.9$ Hz, 1H), 7.36 (d, $J = 7.4$ Hz, 1H), 7.29 (d, $J = 8.1$ Hz, 1H), 4.67 (m, 1H), 4.00 (s, 3H), 2.79 (s, 3H), 2.59 (t, $J = 6.9$ Hz, 2H), 2.16 (p, $J = 7.1$ Hz, 2H), 1.50 (s, 6H). $^{13}$C NMR (101 MHz, DMSO) $\delta$ 194.99, 148.50, 144.60, 131.00, 128.34, 115.27, 112.96, 56.75, 53.99, 50.05, 47.76, 24.66, 22.30, 13.61.

### 2.2 General procedure for the synthesis of 1–4

In a 50-mL round-bottom flask 2,3,3-trimethylindolium-propyl-sulfonates (II1–3) were dissolved in absolute EtOH (ca. 20 mL/g). The corresponding salicylaldehyde (2 equivalents) was added, the flask sealed with a septum and the solution degassed by gentle N$_2$ bubbling (10 min) prior to heating it up to 90 °C under stirring for 24 h. The resulting orange precipitate was filtered, washed thoroughly with EtOH to remove unreacted materials and recrystallized from hot MeOH. Crystals were collected, washed with cold MeOH and dried under vacuum (isolated yield ≤ 50%). X-ray quality crystals can grow also using EtOH as co-solvent or by slow evaporation of NMR samples.

**1.** $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.92 (s, 1H), 8.50 (d, $J = 16.4$ Hz, 1H), 8.24 (d, $J = 7.9$ Hz, 1H), 7.95 (d, $J = 8.8$ Hz, 1H), 7.81 (d, $J = 16.4$ Hz, 1H), 7.51 (d, $J = 2.5$ Hz, 1H), 7.44 (m, 1H), 7.17 (dd, $J = 8.8, 2.5$ Hz, 1H), 7.02 (d, $J = 8.3$ Hz, 1H), 6.97 (t, $J = 7.6$ Hz, 1H), 4.77 (t, $J = 6.8$ Hz, 2H), 3.89 (s, 3H), 2.63 (t, $J = 6.8$ Hz, 2H), 2.16 (p, $J = 6.8$ Hz, 2H), 1.76 (s, 6H). $^{13}$C-NMR (101 MHz, DMSO-$d_6$) $\delta$ 179.48, 160.72, 158.62, 146.73, 145.70, 135.17, 134.18, 129.53, 121.41, 119.99, 116.54, 116.23,
114.86, 111.56, 108.84, 56.13, 51.82, 47.31, 45.58, 26.43, 24.71. HRMS (ESI/QTOF) m/z: [M + H]+ Calcd for C_{22}H_{26}NO_{5}S^{+} 416.1532; Found 416.1528.

2. ^1^H NMR (400 MHz, DMSO-\textit{d}_{6}) \delta 10.96 (s, 1H), 8.57 (d, \textit{J} = 16.3 Hz, 1H), 8.31 (d, \textit{J} = 7.9 Hz, 1H), 7.88 (d, \textit{J} = 16.4 Hz, 1H), 7.57 (t, \textit{J} = 7.9 Hz, 1H), 7.49 – 7.38 (m, 2H), 7.29 (d, \textit{J} = 8.4 Hz, 1H), 7.02 (d, \textit{J} = 8.3 Hz, 1H), 6.96 (t, \textit{J} = 7.5 Hz, 1H), 4.89 (t, \textit{J} = 7.9 Hz, 2H), 4.02 (s, 3H), 2.63 (t, \textit{J} = 6.6 Hz, 2H), 2.21 (p, \textit{J} = 6.1 Hz, 3H), 1.75 (s, 6H). ^1^C NMR (101 MHz, DMSO-\textit{d}_{6}) \delta 180.91, 158.80, 148.65, 147.90, 146.06, 135.45, 130.67, 129.69, 128.27, 121.41, 120.05, 116.51, 114.92, 112.95, 111.30, 56.80, 51.70, 48.78, 47.46, 40.15, 39.94, 39.73, 39.52, 39.31, 39.10, 38.89, 26.83, 25.46. HRMS (ESI/QTOF) m/z: [M + H]+ Calcd for C_{22}H_{26}NO_{5}S^{+} 416.1532; Found 416.1533.

3. ^1^H NMR (400 MHz, DMSO-\textit{d}_{6}) \delta(ppm) 11.34 (s, 1H), 8.64 (d, \textit{J} = 16.3 Hz, 1H), 8.01 (d, \textit{J} = 7.6 Hz, 1H), 7.95 (d, \textit{J} = 16.3 Hz, 1H), 7.85 (d, \textit{J} = 7.0 Hz, 1H), 7.61 (m, 2H), 7.43 (t, \textit{J} = 8.3 Hz, 1H), 6.65 (dd, \textit{J} = 8.4, 3.5 Hz, 2H), 4.64 (t, \textit{J} = 7.2 Hz, 2H), 2.66 (t, \textit{J} = 6.8 Hz, 2H), 2.17 (p, \textit{J} = 7.2 Hz, 2H), 1.76 (s, 6H). ^1^C NMR (101 MHz, DMSO) \delta(ppm) 182.32, 161.48, 160.64, 145.85, 143.19, 141.04, 136.62, 129.11, 128.82, 122.93, 114.87, 112.85, 110.91, 108.66, 102.50, 56.50, 51.55, 47.92, 45.87, 26.77, 24.25. HRMS (ESI/QTOF) m/z: [M + H]+ calcld for C_{22}H_{26}NO_{5}S^{+}, 416.1532 found 415.1534.

4. ^1^H NMR (400 MHz, DMSO-\textit{d}_{6}) \delta 11.29 (s, 1H), 8.54 (d, \textit{J} = 16.1 Hz, 1H), 8.29 (d, \textit{J} = 9.0 Hz, 1H), 7.93 (d, \textit{J} = 7.8 Hz, 1H), 7.82 (d, \textit{J} = 7.3 Hz, 1H), 7.71 (d, \textit{J} = 16.1 Hz, 1H), 7.57 (m, 2H), 6.63 (dd, \textit{J} = 8.8, 2.4 Hz, 1H), 6.55 (d, \textit{J} = 2.5 Hz, 1H), 4.73 (t, \textit{J} = 7.9 Hz, 2H), 2.64 (t, \textit{J} = 6.4 Hz, 2H), 2.15 (p, \textit{J} = 7.7 Hz, 2H), 1.74 (s, 6H). ^1^C NMR (101 MHz, DMSO-\textit{d}_{6}) \delta 181.06, 166.21, 161.70, 148.78, 143.02, 141.02, 131.92, 129.03, 128.40, 122.87, 115.33, 114.44, 108.41, 108.13, 100.63, 55.75, 51.36, 47.37, 44.94, 26.76, 24.35. HRMS (ESI/QTOF) m/z: [M + H]^+ Calcd for C_{22}H_{26}NO_{5}S^{+} 416.1532; Found 416.1528

See Figures S12–27 for the full ^1^H and ^1^C NMR spectra.
3. Crystallographic data summary

| Structure | Compound | CCDC Deposition Number | Empirical formula | Formula weight | Temperature/K | Crystal system | Space group | a/Å | b/Å | c/Å | α/° | β/° | γ/° | Volume/Å³ | Z | ρcalc/g/cm³ | μ/mm⁻¹ | F(000) | χ² | Crystal size/mm³ | Radiation | 2Θ range for data collection | Index ranges | Reflections collected | Independent reflections | Data/restraints/parameters | Goodness-of-fit on F² | Final R indexes [I>2σ(I)] | Final R indexes [all data] | Largest diff. peak/hole / e Å⁻³ |
|-----------|----------|------------------------|-------------------|---------------|---------------|---------------|--------------|------|-----|-----|-----|-----|-----|----------|---|----------------|--------|----------|----------------|-------------------|-----------------------------|----------------|-------------------|-----------------------------|-----------------------------|
| ![Structure 1](image1) | 1 (CSB-27) | 2082614 | C₁₆H₁₅NO₂S | 507.62 (1·2EtOH) | 140.00(10) | triclinic | P-1 | 9.6537(6) | 10.4763(8) | 13.9528(8) | 105.581(6) | 100.221(5) | 101.036(6) | 1294.45(16) | 2 | 1.302 | 1.488 | 544.0 | 0.247 × 0.167 × 0.117 | CuKα (λ = 1.54184) | 9.462 to 146.898 | -11 ≤ h ≤ 8, -12 ≤ k ≤ 12, -14 ≤ l ≤ 17 | 5215 | 5215 [Rsigma = 0.0557] | 5215/68/356 | 0.929 | R₁ = 0.0472, wR₂ = 0.1175 | R₁ = 0.0704, wR₂ = 0.1253 | 0.57/-0.28 |
| ![Structure 2](image2) | 2 (CP-14) | 2082821 | C₁₆H₁₅NO₂S | 493.62 (2·DMSO) | 140.00(10) | triclinic | P-1 | 11.3019(4) | 15.8376(8) | 16.1235(7) | 15.34171(9) | 89.549(3) | 73.3923(3) | 2395.8(2) | 4 | 1.368 | 2.357 | 1048.0 | 0.767 × 0.162 × 0.136 | CuKα (λ = 1.54184) | 6.722 to 147.42 | -16 ≤ h ≤ 15, -17 ≤ k ≤ 17, -16 ≤ l ≤ 16 | 9772 | 9772 [Rsigma = 0.0265] | 9772/0/642 | 0.989 | R₁ = 0.0373, wR₂ = 0.0977 | R₁ = 0.0468, wR₂ = 0.1014 | 0.33/0.41 |
| ![Structure 3](image3) | 3 (CSB-25) | 2082822 | C₁₅H₁₄NO₃S | 465.55 (3·MeOH·H₂O) | 140.00(10) | monoclinic | P2₁/c | 12.49106(8) | 14.34171(9) | 13.24822(8) | 90 | 97.5924(5) | 90 | 2352.52(3) | 4 | 1.314 | 1.591 | 26172 | 0.24 × 0.16 × 0.126 | Cu Kα (λ = 1.54184) | 7.14 to 152.034 | -15 ≤ h ≤ 14, -13 ≤ k ≤ 12, -19 ≤ l ≤ 20 | 26172 | 4894 [Rint = 0.0153, Rsigma = 0.0104] | 4894/82/320 | 1.066 | R₁ = 0.0334, wR₂ = 0.0894 | R₁ = 0.0575, wR₂ = 0.1448 | 0.28/0.32 |
| ![Structure 4](image4) | 4 (CSB-13) | 2081810 | C₁₅H₁₄NO₃S | 433.50 (4·H₂O) | 139.99(10) | monoclinic | P2₁/n | 12.2487(5) | 10.7396(3) | 16.1896(5) | 90 | 92.984(3) | 90 | 2126.79(13) | 4 | 1.354 | 1.686 | 920.0 | 0.355 × 0.261 × 0.028 | CuKα (λ = 1.54184) | 8.836 to 152.376 | -15 ≤ h ≤ 14, -13 ≤ k ≤ 12, -19 ≤ l ≤ 20 | 20471 | 4438 [Rint = 0.0504, Rsigma = 0.0385] | 4438/0/286 | 1.039 | R₁ = 0.0504, wR₂ = 0.1363 | R₁ = 0.0575, wR₂ = 0.1448 | 0.48/-0.51 |

 crystals structures are illustrated with thermal ellipsoids at 50% probability; solvent molecules and hydrogens are omitted for clarity. Note: even if all compounds crystalize in their all trans-MCH conformation (TTT), such conformation is not the only one present in solution.²
4. Hydrolysis

The model was derived as follows. Assuming: i) MCH dissociating into MC, SP and H\(^+\) according to the corresponding ground state acidity constant \((pK_{a}^{GS})\), ii) MCH undergoing reaction with either \(H_2O\) or the hydroxide ion and iii) \(X\) the tetrahedral intermediate leading to products:

\[
\begin{align*}
\text{MCH} & \overset{K_a^{GS}}{\longrightarrow} \text{SP} + \text{MC} + \text{H}^+ \\
\text{MCH} + \text{H}_2\text{O} & \overset{k_w}{\longrightarrow} \overset{k_w}{\longleftarrow} \text{X} + \text{H}^+ \\
\text{MCH} + \text{OH}^- & \overset{k_{OH}}{\longrightarrow} \overset{k_{OH}}{\longleftarrow} \text{X} \\
\text{X} & \overset{k_h}{\longrightarrow} \text{P}
\end{align*}
\]

And considering steady-state conditions in respect to intermediate \(X\):

\[
\frac{d[X]}{dt} \approx 0 = k_w\text{[MCH]} - k_w\text{[X][H}^+] + k_{OH}\text{[MCH][OH}^-] - k_{-OH}\text{[X]} - k_h\text{[X]}
\]

It follows that:

\[
[X] = \frac{[\text{MCH}](k_w + k_{OH}[\text{OH}^-])}{k_w[H^+] + k_{-OH} + k_h}
\]

Given the initial rate equal to \(v_0 = k_h[X]\) and expressing \([\text{MCH}]\) as a function of \(C\) and \(pK_{a}^{GS}\):

\[
[\text{MCH}] = \frac{C[H^+]}{[H^+] + K_{a}^{GS}}
\]

We finally obtain:

\[
v_0 = k_h \cdot X = \frac{k_hC[H^+](k_w + k_{OH}[\text{OH}^-])}{([H^+] + K_{a}^{GS})(k_w[H^+] + k_{-OH} + k_h)} = \frac{C(k_w[H^+] + k_{OH}K_w)}{([H^+] + K_{a}^{GS})\left(\frac{k_w}{k_h}[H^+] + \frac{k_{-OH}}{k_h} + 1\right)}
\]

And so (realizing that \(k_{OH}/k_h << 1\)):

\[
k_{obs,hydr} \approx \frac{v_0}{C} = \frac{k_w[H^+] + k_{OH}K_w}{([H^+] + K_{a}^{GS})\left(\frac{k_w}{k_h}[H^+] + 1\right)}
\]

\text{eqn (S1)}
Figure S1. Top: linearized kinetic profiles for the hydrolysis of 1, 2, 3 and 4 at different pH in the dark (duplicate experiments). Middle: plots of the obtained apparent first order rate constant of hydrolysis as a function of the pH and relative fitting; solid black lines represent the best fit to eqn S1 above. Bottom: Comparison of the obtained kinetic parameters with those of Liao’s photoacid (see ref. S1). Experimental conditions: [1–4] = 25 ± 2 μM, [phosphate buffers] = 20 mM (3 < pH < 8), T = 25 °C; data collected the maximum of the corresponding MCH (3 < pH < 7) and MC form (pH > 7).

| Compound | Liao | 1     | 2     | 3     | 4     |
|----------|------|-------|-------|-------|-------|
| pK_a^{GS} | 6.20 | 6.95  | 6.81  | 5.96  | 6.37  |
| k_w (min⁻¹) | 0.00308 | 0.0059 | 0.0069 | 0.00126 | 0.00094 |
| k_wk_h⁻¹ (M⁻¹) | 7.5E+05 | 4.7E+05 | 3.2E+05 | 2.8E+05 | 1.1E+06 |
| k_OH (M⁻¹min⁻¹) | 9.3E+03 | 2.2E+03 | 2.5E+03 | 7.6E+03 | 4.8E+03 |
Figure S2. Representative UV-Vis spectra as a function of time (left) and corresponding kinetic profile (right) after on-site addition and rapid mixing of an aliquot (50 μL) of a solution (0.5 mM in mQ water) of compound 1 (top) and 4 (bottom) into a cuvette containing the buffer solution (950 μL); in the case of 1, the solid black line represents the best fit to a first-order model equation. $K_c$, $k_2$ and $k_{-2}$ values and relative error refer to the same experiment performed in duplicate. Experimental conditions: [1-4] = 25 ± 2 μM, [phosphate buffer] = 20 mM, pH 9.5, $T = 25$ °C.
6. SP-to-MC(H) relaxation in the dark

Figure S3. Kinetic profiles for the relaxation of 1 and 4 at pH 5.2 after light irradiation (425 nm, 100 mW); solid black lines represent the best fit to a first-order model equation. In the case of 1, the $k_2$ value and relative error refers to the same experiment performed in triplicate. Experimental conditions: [1-4] = 25 ± 2 μM, [phosphate buffer] = 20 mM, pH 5.2, T = 25 °C

The total concentration of photoacid is always expressed by the sum of all the four species in solution:

$$C = [cMCH] + [MCH] + [MC] + [SP]$$

Considering cis-MCH (indicated in equations with the short label cMCH for simplicity) produced exclusively by photoirradiation it follows that the total concentration in the dark can be reasonably approximated as:

$$C = C^{dark} \approx [MCH]^{eq} + [MC]^{eq} + [SP]^{eq}$$

Instead, under light irradiation (with both MCH and MC converted into cis-MCH and/or SP), the expression for $C$ becomes:

$$C = C^{hv} = [cMCH]^{hv} + [SP]^{hv} + [SP]^{eq} = [cMCH]_0 + [SP]_0$$

This can be reformulated in terms of [SP] considering the acid-base equilibrium existing between cis-MCH and SP:

$$C = C^{hv} = [SP]_0 \left( 1 + \frac{[H^+]}{K_{aMCH}} \right)$$
On the other hand, the effective concentration of photochemically-populated metastable species \( C_{0}^{hv} \) can be obtained as:

\[
C^{hv} - C^{dark} = [cMCH]^{hv} + [SP]^{hv} - [MCH]^{eq} - [MC]^{eq} = 0
\]

\[
C_{0}^{hv} = [cMCH]^{hv} + [SP]^{hv} = [MCH]^{eq} + [MC]^{eq} = C(1 - \chi_{SP})
\]

Where \( \chi_{SP} \) is the molar fraction of SP at equilibrium under dark conditions. On multiplying both sides of the equation above for \( C^{hv} \) by \((1 - \chi_{SP})\), \( C_{0}^{hv} \) can be reformulated as:

\[
C_{0}^{hv} = C(1 - \chi_{SP}) = [SP]_{0} \left( 1 + \frac{[H^{+}]}{K_{a}^{MS}} \right)(1 - \chi_{SP})
\]

This equation directly relates with the pre-equilibrium between \( cis \)-MCH and SP – i.e., with the total concentration of the species after light irradiation – and allows for extrapolating the concentration of SP at \( t = 0 \) and any pH as:

\[
[SP]_{0} = \frac{C_{0}^{hv}}{(1 - \chi_{SP})} \left( \frac{K_{a}^{MS}}{[H^{+}] + K_{a}^{MS}} \right)
\]

Considering now the ring-opening of SP towards MC the rate-determining step throughout the entire range of pH:

\[
cis\text{-MCH} \quad K_{a}^{MS} \quad \text{SP} + H^{+}
\]

\[
\text{SP} \quad k_{-2} \quad \text{P}
\]

It follows that:

\[
v_{0} \approx k_{-2} [SP]_{0} = k_{-2} \frac{C_{0}^{hv}}{(1 - \chi_{SP})} \left( \frac{K_{a}^{MS}}{[H^{+}] + K_{a}^{MS}} \right)
\]

And so:

\[
k_{obs,relax} \approx \frac{v_{0}}{c_{0}^{hv}} = \frac{k_{-2}}{(1 - \chi_{SP})} \left( \frac{K_{a}^{MS}}{1 + K_{a}^{MS}} \right)
\]

Finally, expressing \((1 - \chi_{SP})\) as a function of all equilibrium constants in the dark, we obtain the rate expression for the thermal relaxation as:

\[
k_{obs,relax} = k_{-2} \left( \frac{K_{a}^{MS}}{[H^{+}] + K_{a}^{MS}} \right) \left( \frac{K_{a} + K_{C}}{1 + [H^{+}] + K_{a}} \right) \quad \text{eqn (S2)}
\]
Equation S2 suits the double sigmoidal profiles obtained below, with conditions for plateau and inflections extrapolated as follow:

\[
K_a^{MS} \ll [H^+] \gg K_a \quad \Rightarrow \quad k_{obs,relax} = k_{-2} \left( \frac{K_a^{MS}}{[H^+]} \right)
\]

\[
K_a^{MS} = [H^+] \gg K_a \quad \Rightarrow \quad k_{obs,relax} = k_{-2} \frac{2}{2}
\]

\[
K_a^{MS} \gg [H^+] \gg K_a \quad \Rightarrow \quad k_{obs,relax} = k_{-2}
\]

\[
K_a^{MS} \gg [H^+] = K_a \quad \Rightarrow \quad k_{obs,relax} = k_{-2} \left( \frac{2 + K_c}{2} \right)
\]

\[
K_a^{MS} \gg [H^+] \ll K_a \quad \Rightarrow \quad k_{obs,relax} = k_{-2} \left( 1 + K_c \right) = k_{-2} + k_2
\]

The guided graphics in Figure S4 result from these considerations.

Figure S4. In the case of compound 1, the same experiment above is repeated as a function of the pH in duplicate; the solid black line represents the best fit to eqn S2. Fitted values are in good agreement with those determined independently through UV-Vis and $^1$H NMR measurements (see Figures S2, S5 and S6). Together with the corresponding one of hydrolysis (see Figure S1), this profile is used to fit the pH jumps obtained with 1 below.
7. $^1$H NMR titration of 1 in the dark

![Diagram of molecular structures]

Figure S5. (a) Three-component equilibrium system describing the ground state of 1 (top) and $^1$H NMR (600 MHz) spectra at increasing pH values (bottom). Profiles of the chemical shift of a,a' (b) and the integral ratio between c* and c,c' (c) as a function of the pH; solid black lines represent the best fit to the corresponding model equation (see ref. S1 pp. 8459). Experimental conditions: [1] = 0.1 mM, [phosphate buffers] = 20 mM (3 < pH < 10) in H$_2$O/D$_2$O 6:4, $T = 25 \, ^\circ$C
7. Apparent acid dissociation constants of 1 under light irradiation

Figure S6. UV-Vis pH titration (left) and corresponding elaboration (right) obtained using 425 nm (top) and 500 nm (bottom) LED light sources (100 mW). Experimental conditions: [1] = 25 ± 2 μM, [buffers] = 20 mM (pH > 3), T = 25 °C.

pK<sub>a</sub><sup>MS</sup> = 3.43 ± 0.04

pK<sub>a</sub><sup>MS</sup> = 3.31 ± 0.04
8. Molar extinction coefficient of 1-MCH ($\varepsilon_{\text{MCH}}$)

![Graph showing representative spectra as a function of the concentration of 1 at equilibrium in the dark (left) and calibration curve (right). The obtained value and relative error result from five independent experiments. Experimental conditions: [buffer] = 20 mM, pH = 3, $T = 25^\circ\text{C}$.](image)

**Figure S7.** Representative spectra as a function of the concentration of 1 at equilibrium in the dark (left) and calibration curve (right). The obtained value and relative error result from five independent experiments. Experimental conditions: [buffer] = 20 mM, pH = 3, $T = 25^\circ\text{C}$. 

$\varepsilon_{\text{MCH}} = 30350 \pm 290 \text{ M}^{-1}\text{cm}^{-1}$

$R^2 = 0.9999$
8. pH-jump studies

Saturated solutions of 1 in aqueous KCl (30 mL, 20 mM) were prepared by sonication as described previously. Each experiment is run independently, transferring the resulting solution into a 50-mL jacketed beaker kept at 25 °C, equipped with a 2.5 cm cross-shaped stir bar and mounted above a 45° mirror cage. pH data acquisition is carried out under stirring (250 rpm) and inert atmosphere (gentle N₂ bubbling) with a pre-calibrated Unitrode Pt1000 glass electrode (Scheme S2).

Scheme S2. Our photochemical apparatus designed for performing pH jump studies. (1) Metrohm Unitrode Pt1000 glass electrode, (2) 50-mL jacketed beaker connected to a thermostat, (3) fiber-coupled LED-light source ending with a 2.5 cm collimator (a) mounted on a 45° mirror cage (b), (4) nitrogen inlet (needle), (5) satd. solution of photoacid equipped with a cross-shaped stir bar and (6) stir plate. During measurements, an aluminum foil is wrapped around the apparatus for preventing the loss of irradiation.

Concomitantly, the concentration of each satd. solution is determined from optical readings at pH 3 knowing εMCH (see Figure S7).

Note: solubility is extremely sensitive to preparation time, position of the sample in the sonicator, sonicator type, as well as temperature. For this reason we developed an alternative way to determine S_MCH (see sections below). For the following pH jumps, however, each solution was prepared with the old¹ procedure.
Figure S8. Simultaneous fit of three independent variable-power pH jump experiments (top) together with the pH-dependent profiles of relaxation (Figure S6) and hydrolysis (Figure S1) (bottom). Fitting curves are highlighted in red. Fitted parameters are listed in Table S1-3 below for comparison.
Table S1. Fitted parameters for experiment #1 (425 nm light irradiation)

| Parameter | Predicted value | Experimental value | Deviation (\(\sigma\)) |
|-----------|----------------|--------------------|------------------------|
| \(C\)     | 0.000282 \(M\) | 0.000287 \(M\)    | 0.17                   |
| \(pK_a = -\log_{10} K_a\) | 7.32          | 7.4                | 0.5                    |
| \(pK_c = -\log_{10} K_c\) | −0.17         | −0.17              | 0                      |
| \(pK_a^{MS} = -\log_{10} K_a^{MS}\) | 3.29          | 3.31               | 0.06                   |
| \(k_1\)  | 0.061 s\(^{-1}\) | n/a               | n/a                    |
| \(k_2\)  | 0.0066 s\(^{-1}\) | 0.0065 s\(^{-1}\) | 0.15                   |
| \(k_3\)  | 66.7 s\(^{-1}\) | n/a               | n/a                    |
| \(k_4\)  | 4.87 \times 10^{-5} s\(^{-1}\) | n/a               | n/a                    |
| \(k_v (340 mW)\) | 0.0557 s\(^{-1}\) | n/a               | n/a                    |
| \(k_v (125 mW)\) | 0.0199 s\(^{-1}\) | n/a               | n/a                    |
| \(k_v (45 mW)\) | 0.00836 s\(^{-1}\) | n/a               | n/a                    |
| \(k_{OH}\)  | 40.9 s\(^{-1}\) | n/a               | n/a                    |
| \(k_{-OH}\) | 0.00486 s\(^{-1}\) | n/a               | n/a                    |
| \(k_h\)  | 0.239 s\(^{-1}\) | n/a               | n/a                    |
| \(k_{-H}\) | 3.3 \times 10^{-11} s\(^{-1}\) | n/a | n/a                    |
| \(k_w\)  | 1.04 \times 10^{-5} s\(^{-1}\) | 9.83 \times 10^{-6} s\(^{-1}\) | ok\(^b\) |
| \(k_{-w}\) | 123.6 \times 10^{-3} M\(^{-1}\) s\(^{-1}\) | n/a | n/a                    |
| \(k_{-w}/k_h\) | 4.7 \times 10^5 M\(^{-1}\) | 4.7 \times 10^5 M\(^{-1}\) | ok\(^b\) |
| \(pH_{dark}\) | 5.24          | 5.25               | 0.04                   |
| \(pH_{light (340 mW)}\) | 3.74          | 3.73               | 0.05                   |
| \(pH_{light (125 mW)}\) | 3.78          | 3.74               | 0.11                   |
| \(pH_{light (45 mW)}\) | 3.84          | 3.77               | 0.19                   |
| \(\Phi_{425 (340 mW)}\) | 0.39          | n/a               | n/a                    |
| \(\Phi_{425 (125 mW)}\) | 0.38          | n/a               | n/a                    |
| \(\Phi_{425 (45 mW)}\) | 0.44          | n/a               | n/a                    |
| \(\Phi_{425 (Mean \pm St. Dev.)}\) | 0.40 \pm 0.03 | n/a               | n/a                    |

\(a\). average values obtained considering both UV-Vis and \(^1\)H NMR, or pH jump experiments. In all cases, error stays within 10%.

\(b\). kinetic constants for hydrolysis are optimized esteem resulting from fitting to the equation S1, so checked for the order of magnitude to be consistent.
### Table S2. Fitted parameters for experiment #2 (425 nm light irradiation)

| Parameter               | Predicted value | Experimental value | Deviation (σ) |
|-------------------------|-----------------|--------------------|---------------|
| $C$                     | 0.000336 $M$    | 0.000291 $M$       | 1.55 $^{c}$   |
| $k_v$ (340 mW)          | 0.0469 s$^{-1}$ | n/a                | n/a           |
| $k_v$ (125 mW)          | 0.0165 s$^{-1}$ | n/a                | n/a           |
| $k_v$ (45 mW)           | 0.00672 s$^{-1}$| n/a                | n/a           |
| $pH_{\text{dark}}$      | 5.20            | 5.18               | 0.04          |
| $pH_{\text{light}}$ (340 mW) | 3.69          | 3.74               | 0.13          |
| $pH_{\text{light}}$ (125 mW) | 3.72          | 3.75               | 0.08          |
| $pH_{\text{light}}$ (45 mW) | 3.80          | 3.78               | 0.05          |
| $\Phi_{425}$ (340 mW)   | 0.39            | n/a                | n/a           |
| $\Phi_{425}$ (125 mW)   | 0.37            | n/a                | n/a           |
| $\Phi_{425}$ (45 mW)    | 0.42            | n/a                | n/a           |
| $\Phi_{425}$ (Mean ± St. Dev.) | 0.40 ± 0.02 | n/a                | n/a           |

$c$. This deviation was likely due to a pipetting error during UV-Vis concentration check.

### Table S3. Fitted parameters for experiment #3 (500 nm light irradiation)

| Parameter               | Predicted value | Experimental value | Deviation (σ) |
|-------------------------|-----------------|--------------------|---------------|
| $C$                     | 0.000210 $M$    | 0.000216 $M$       | 0.28          |
| $k_v$ (195 mW)          | 0.049 s$^{-1}$  | n/a                | n/a           |
| $k_v$ (135 mW)          | 0.0347 s$^{-1}$ | n/a                | n/a           |
| $k_v$ (85 mW)           | 0.0218 s$^{-1}$ | n/a                | n/a           |
| $k_v$ (45 mW)           | 0.013 s$^{-1}$  | n/a                | n/a           |
| $pH_{\text{dark}}$      | 5.31            | 5.31               | 0             |
| $pH_{\text{light}}$ (195 mW) | 3.85          | 3.85               | 0             |
| $pH_{\text{light}}$ (135 mW) | 3.86          | 3.85               | 0.03          |
| $pH_{\text{light}}$ (85 mW) | 3.88          | 3.86               | 0.05          |
| $pH_{\text{light}}$ (45 mW) | 3.91          | 3.87               | 0.1           |
| $\Phi_{500}$ (195 mW)   | 0.38            | n/a                | n/a           |
| $\Phi_{500}$ (135 mW)   | 0.39            | n/a                | n/a           |
| $\Phi_{500}$ (85 mW)    | 0.39            | n/a                | n/a           |
| $\Phi_{500}$ (45 mW)    | 0.43            | n/a                | n/a           |
| $\Phi_{500}$ (Mean ± St. Dev.) | 0.40 ± 0.03 | n/a                | n/a           |
8.1 Preparation of buffered solutions

Saturated solutions of 1 are prepared by stirring (250 rpm) an excess amount of grinded crystals into aqueous KCl (15 mL, 20 mM) containing HCl ($C_{HCl} = 1$ mM $\rightarrow S_{MCH}$) or increasing amounts of NaOH ($C_{NaOH} = \alpha \cdot S_{MCH}$, $0 < \alpha \leq 1 \rightarrow S_{tot}$). We used the same apparatus described above in Scheme S2 to keep the temperature constant ($T = 25$ °C) and monitor the pH during dissolution in the dark. In all cases, the pH stabilizes within 20 minutes (Figure S9). The resulting mixtures are then microfiltered (H-PTFE-45/25) and subjected to UV-Vis (Figure S10) prior to pH jump analyses (Figure S11).

![Figure S9](image-url)

**Figure S9.** Time course of pH upon solubilization of compound 1 in aqueous KCl containing HCl (red trace) or increasing amounts of NaOH (0.1 N, 11, 22, 33, 44 and 55 μL, from bottom to top respectively). The amount of NaOH was calculated with respect to $S_{MCH}$ in order to cover the range $0 < \alpha \leq 1$. Grounded crystals of 1 were added after 100 seconds.
8.2 Solubility vs. $\alpha$

Figure S10. Representative spectra as a function of $\alpha$ after equilibration (20 min) in the dark (left); dilution factor was 20: 50 $\mu$L of PTFE-filtered sample solution, final volume = 1.0 mL; Absorbances at 437 nm are converted into concentration knowing $\varepsilon_{\text{MCH}}$ (see Figure S7). Solubility of 1 vs. alpha (right); solid black line represents the best fit to eqn 1 in the main text; the resulting $S_{\text{MCH}}$ value is in agreement with the one obtained after solubilization in HCl 1 mM: 0.37 ± 0.3 mM (triplicate experiment). Experimental conditions: [buffer] = 20 mM, pH = 4.5, $T = 25^\circ C$. 

$S_{\text{MCH}} = 0.36$ mM

$\varepsilon_{\text{MCH}}$

$\alpha$

$A_{\text{eq}}$

wavelength / nm

 equivalents of NaOH ($\alpha$)

$S_{\text{TOT}}$ / mM

STOT / mM

SMCH = 0.36 mM

$A_{\text{eq}}$

$\alpha$

$0.0$

$0.4$

$0.8$

$1.2$

$0.0$

$0.4$

$0.8$

$1.2$

$200$

$300$

$400$

$500$

$600$

$700$

$0$

$0.3$

$0.6$

$0.9$

$0$

$0.2$

$0.4$

$0.6$

$0.8$

$1$

$200$

$300$

$400$

$500$

$600$

$700$

$0$

$0.3$

$0.6$

$0.9$
8.3 pH jumps vs. α

Figure S11. From left to right: pH jumps experiments, ΔpH = pH_{dark} – pH_{light} and pH_{dark} as a function of α. Solid red lines represent model predictions of our four-state cycle taking into account the degree of neutralization in the dark, whereas the solid black lines represent the best fit to eqn 2 in the main text. The resulting pK_{a}^{GS} is in line with the expected one. Experimental conditions: [I]_{0} = see Figure S10, T = 25 °C.
8. $^1$H and $^{13}$C NMR spectra

Figure S12. $^1$H NMR spectrum (400 MHz, DMSO-$d_6$) of I1.

Figure S13. $^{13}$C NMR spectrum (101 MHz, DMSO-$d_6$) of I1.
Figure S14. $^1$H NMR spectrum (400 MHz, CDCl$_3$) of I2.

Figure S15. $^{13}$C NMR spectrum (101 MHz, CDCl$_3$) of I2.
Figure S16. $^1$H NMR spectrum (400 MHz, DMSO-$d_6$) of III.

Figure S17. $^{13}$C NMR spectrum (101 MHz, DMSO-$d_6$) of III.
Figure S18. $^1$H NMR spectrum (400 MHz, DMSO-$d_6$) of II2.

Figure S19. $^{13}$C NMR spectrum (101 MHz, DMSO-$d_6$) of II2.
Figure S20. $^1$H NMR spectrum (400 MHz, DMSO-$d_6$) of 1.

Figure S21. $^{13}$C NMR spectrum (101 MHz, DMSO-$d_6$) of 1.
Figure S22. $^1$H NMR spectrum (400 MHz, DMSO-$d_6$) of 2.

Figure S23. $^{13}$C NMR spectrum (101 MHz, DMSO-$d_6$) of 2.
Figure S24. $^1$H NMR spectrum (400 MHz, DMSO-$d_6$) of 3.

Figure S25. $^{13}$C NMR spectrum (101 MHz, DMSO-$d_6$) of 3.
Figure S26. $^1$H NMR spectrum (400 MHz, DMSO-$d_6$) of 4.

Figure S27. $^{13}$C NMR spectrum (101 MHz, DMSO-$d_6$) of 4.
9. References

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