Supporting Information

pH-responsive carborhodol-coupled chitosan-based hydrogels for the detection of wound infection-relevant pH changes

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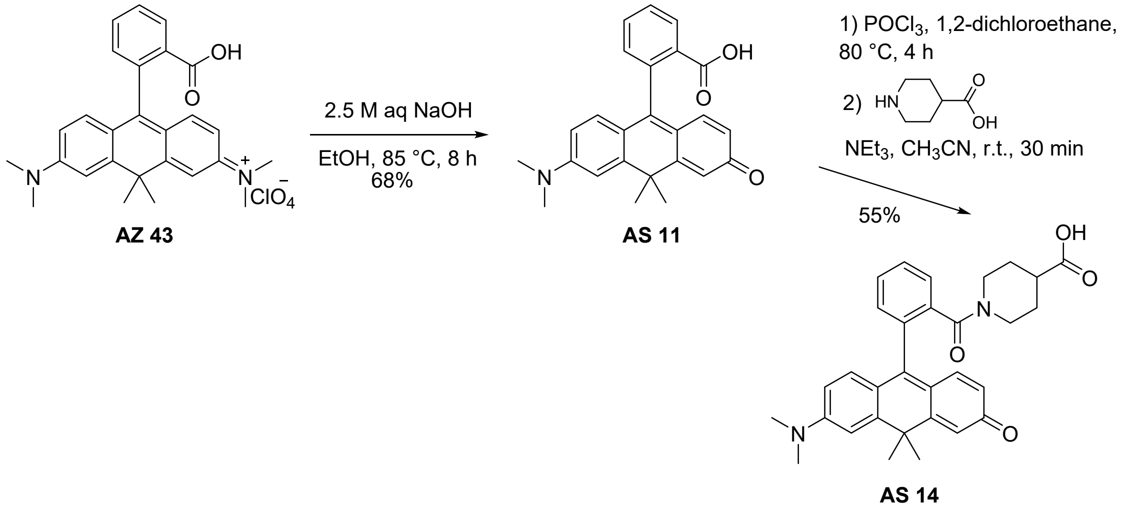
1. Synthesis of novel pH-responsive carborhodol derivatives

1.1. Synthesis of AS 14 via AS 11

1.1.1. 2-(6-(Dimethylamino)-10,10-dimethyl-3-oxo-3,10-dihydroanthracen-9-yl)benzoic acid (AS 11) synthesis [1]

AZ 43 (0.40 g, 0.78 mmol) was dissolved in a 1:1 mixture of ethanol (50 mL) and 2.5 M aq. NaOH (50.0 mL, 125 mmol). The reaction mixture was stirred at 85 °C for 8 h (Scheme S1, conversion checked by HPLC). The color of the solution changed from blue to red within 30 minutes. After the completion of the reaction, the reaction mixture was cooled to 0 °C and acidified to pH 5 using TFA. The reaction mixture was extracted with DCM (60 mL), resulting in three layers (DCM, EtOH-rich, and aqueous). The DCM layer was collected, and the remaining phases were extracted with DCM (60 mL). Brine (20 mL) was added to aid separation, and the aqueous layer was further extracted with DCM (3 × 60 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by reverse phase column chromatography (C18 modified SiO_2, 120 g; H_2O/MeCN/TFA 7/3/0.001). The red-colored fractions were collected and concentrated under reduced pressure, yielding AS 11 as red powder (202 mg, 524 µmol, 67%).



Scheme S1: AS 14 synthesis.

AS 11 HPLC: CH_3CN/H_2O + 0.1 v/v% TFA = 15/85 to 95/5 in 30 min, detection at 550 nm, t_R = 8.48 min (>99%).

AS 11 ^{1}H-NMR (500 MHz, DMSO-d_6): δ = 1.67 (s, 3 H, 10-CH_3), 1.78 (s, 3 H, 10-CH_3’), 2.93 (s, 6 H, 6-$N(CH_3)_2$), 6.44 (d, ^3J = 9 Hz, 1 H, 8-H), 6.47 (d, ^3J = 9 Hz, 1 H, 1-H), 6.59 (dd, ^3J = 9 Hz, ^4J = 2 Hz, 1 H, 7-H), 6.61 (dd, ^3J = 9 Hz, ^4J = 2 Hz, 1 H, 2-H), 6.92 (d, ^4J = 2 Hz, 1 H, 5-H), 7.06 (d, ^3J = 8 Hz, 1 H, 6’-H), 7.09 (d,^4J = 2 Hz, 1 H, 4-H), 7.64 (ddd, ^3J = 7 Hz, ^4J = 2 Hz, 1 H, 4’-H), 7.70 (ddd, ^3J = 7 Hz, ^4J = 2 Hz, 1 H, 5’-H), 7.96 (dd, ^3J = 8 Hz, ^4J = 2 Hz, 1 H, 3’-H), 9.67 (br. s, 1H, 2’-COOH) (Fig. S1).

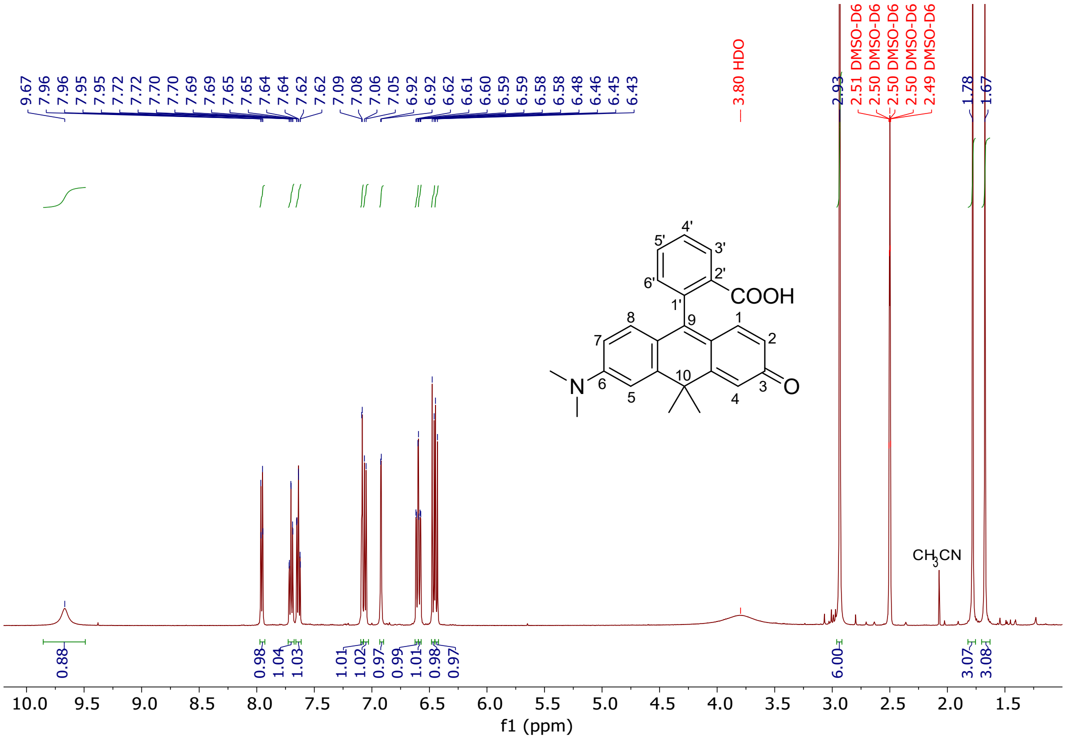


Figure S1. ^{1}H-NMR spectrum (500 MHz) of AS 11 in DMSO-d_6.

AS 11 ^{13}C-NMR (125 MHz, DMSO-d_6): δ = 33.0 (10-CH$_3$), 34.5 (10-CH$_3$’), 37.8 (C10), 39.6 (6-N(CH$_3$)$_2$), 109.0 (C5),111.9 (C7), 112.6 (C4),114.7 (C2), 118.5 (C8a), 121.8 (C9a), 123.6 (C6’) 124.5 (C3’), 125.7 (C2’), 128.1 (C8), 128.7 (C1), 128.7 (C9), 129.3 (C4’), 135.2 (C5’), 145.6 (C10a), 147.0 (C4a),150.5 (C6), 155.1 (C1’), 157.9 (C3), 169.8 (2’-COOH) (Fig. S2).

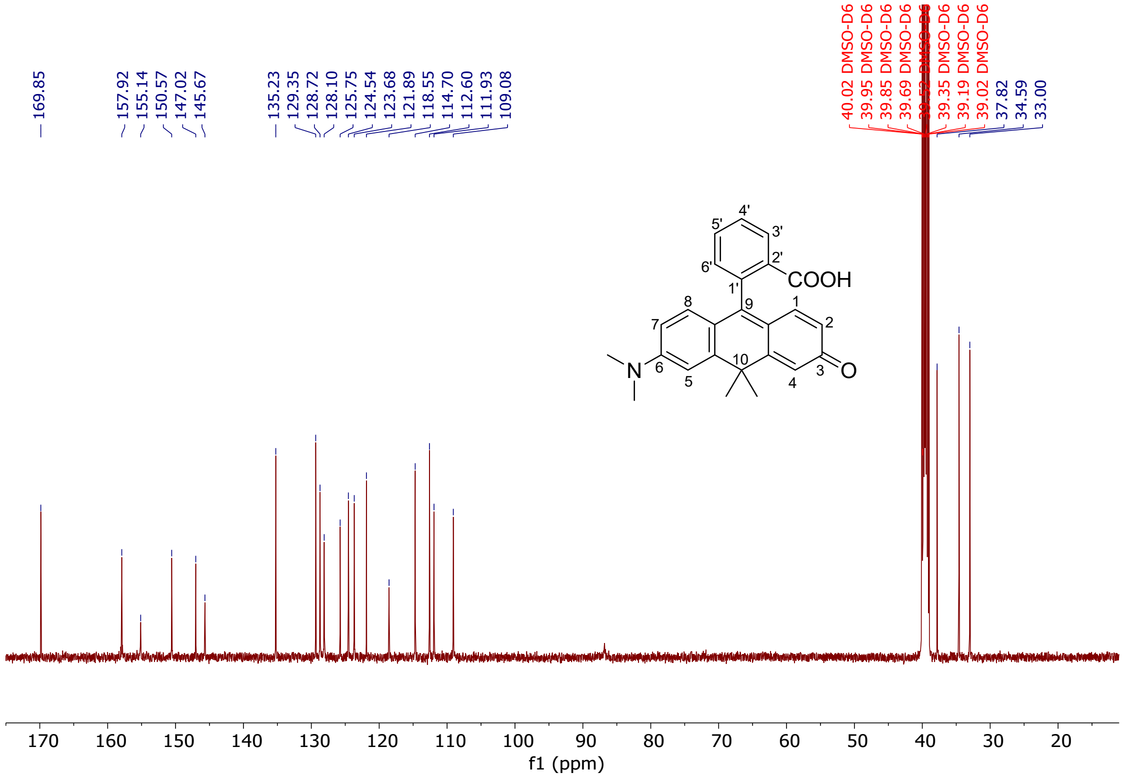


Figure S2. ^{13}C-NMR spectrum (125 MHz) of AS 11 in DMSO-d_6.

AS 11 LCMS (ESI$^+$): *m*/*z* calcd for $C_{25}H_{23}NO_3$ [M + H]$^+$: 386.2, found 386.2.

1.1.2. 1-(2-(6-(Dimethylamino)-10,10-dimethyl-3-oxo-3,10-dihydroanthracen-9-yl)benzoyl) piperidine-4-carboxylic acid (AS 14) synthesis

To a solution of AS 11 (100 mg, 0.2 mmol) in 1,2-dichloroethane (10 mL), $POCl_3$ (951 µL, 10.2 mmol) was added (Scheme S1). After stirring at 80 °C for 4 h, all volatile materials were evaporated *in vacuo*, and the solid residue was dissolved in CH_3CN (5 mL). A solution of piperidine-4-carboxylic acid (129 mg, 1.00 mmol) in CH_3CN (5 mL) was added, followed by the addition of NEt_3 (842 µL, 6.00 mmol). After stirring at room temperature for 30 min, all volatile materials were removed *in vacuo*. The residue was redissolved in DCM (20 mL) and washed with a saturated aq. NH_4Cl solution. The organic layer was separated, dried with anhydrous Na_2SO_4, and the solvent was removed under reduced pressure. Reverse phase column chromatography (C18 modified SiO_2, 40 g; $H_2O/CH_3CN/TFA$ 8/2/0.001) yielded AS 14 (67 mg, 0.11 mmol, 55%) as a red solid.

AS 14 HPLC: CH_3CN/H_2O + 0.1 v/v% TFA = 15/85 to 95/5 in 60 min, detection at 550 nm, t_R = 11.97 min (97%).

AS 14 ^{1}H-NMR (500 MHz, CD_3OD): δ = 1.23–1.34 (m, 4 H, CH_2’), 1.67 (s, 3 H, 10-CH_3), 1.84 (s, 3 H, 10-CH_3’), 2.41–2.48 (m, 1 H, CH_2), 2.66–2.71 (m, 1 H, CH), 2.94–3.03 (m, 1 H, CH_2), 3.49 (s, 6 H, 6-$N(CH_3)_2$), 3.56–3.70 (m, 1 H, CH_2), 4.06–4.14 (m, 1 H, CH_2), 6.78 (dd, ^3J = 9 Hz, ^4J = 2 Hz, 1 H, 2-H), 7.01 (dd, ^3J = 9 Hz, ^4J = 2 Hz, 1 H, 7-H), 7.20 (d, ^3J = 9 Hz, 1 H, 1-H), 7.30 (d, ^4J = 2 Hz, 1 H, 4-H), 7.33 (d, ^3J = 7 Hz, 1 H, 8-H), 7.34 (s, 1 H, 5-H), 7.45–7.47 (m, 1 H, 3’-H), 7.56–7.60 (m, 1 H, 6’-H), 7.70–7.74 (m, 2 H, 4’-H & 5’-H) (Fig. S3).

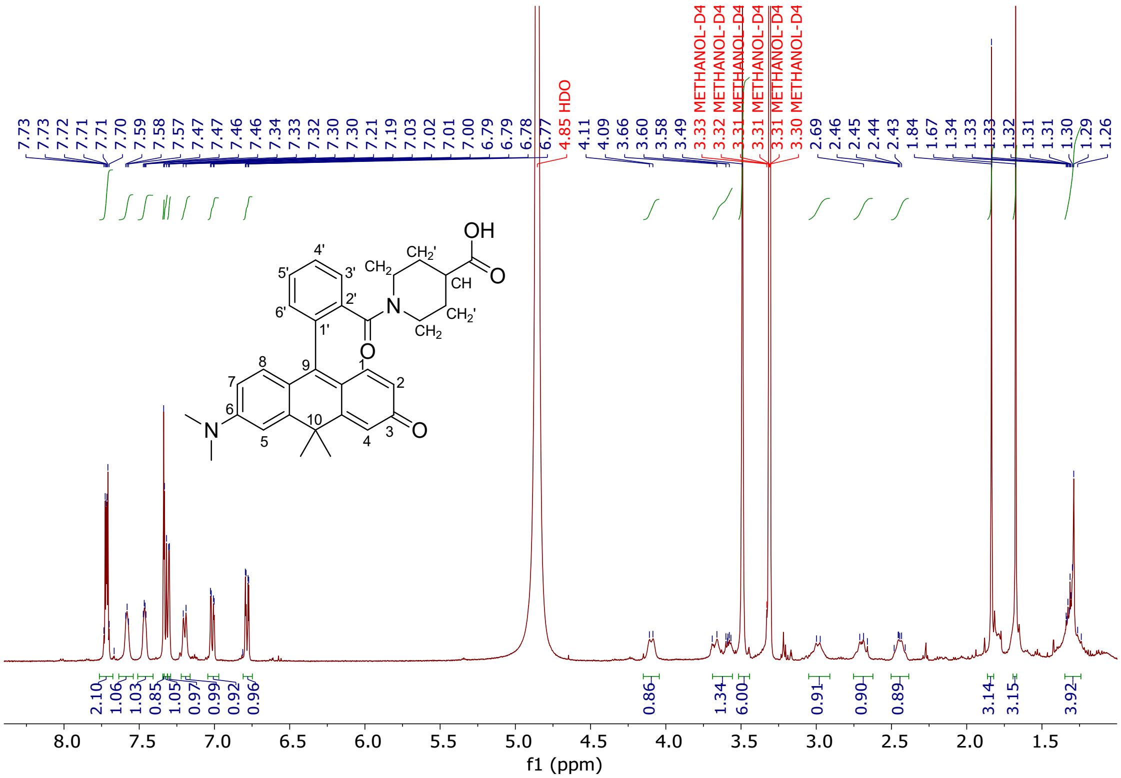


Figure S3. ^{1}H-NMR spectrum (500 MHz) of AS 14 in CD_3OD.

AS 14 ^{13}C-NMR (125 MHz, CD_3OD): δ = 30.7 (CH_2’), 31.2 (10-CH_3), 31.2 (10-CH_3), 36.1 (10-CH_3’), 41.4 (CH_2), 41.9 (6-$N(CH_3)_2$), 42.1 (CH), 42.9 (C10), 113.8 (C5), 115.8 (C7), 116.3 (C4), 117.1 (C8a), 117.4 (C2), 117.7 (C9a), 123.7 (C9), 128.2 (C6’), 130.5 (C4’ & C5’), 130.7 (C1’), 131.6 (C3’), 137.0 (C2’), 138.6 (C1), 140.9 (C8), 157.0 (C4a), 159.9 (C6), 160.6 (C10a), 163.3 (C2’-$\underline{C}ONC_5H_9COOH$), 168.8 (C3), 177.6 (C2’-$CONC_5H_9\underline{C}OOH$) (Fig. S4).

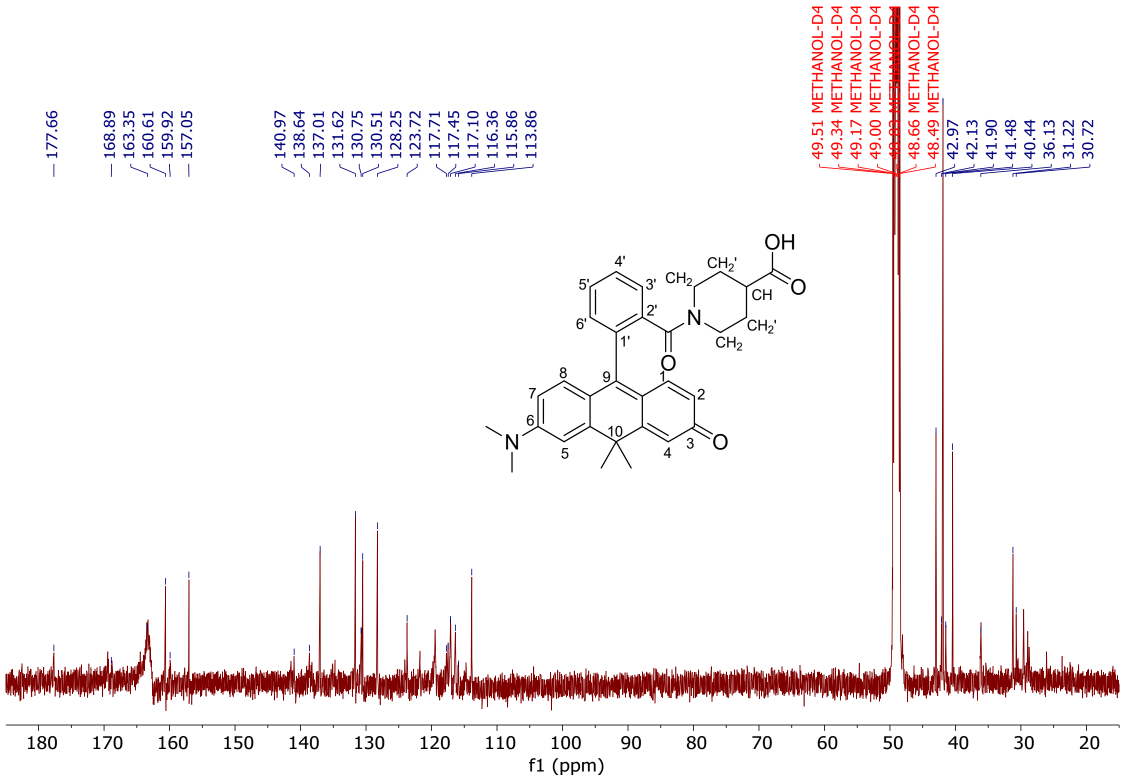


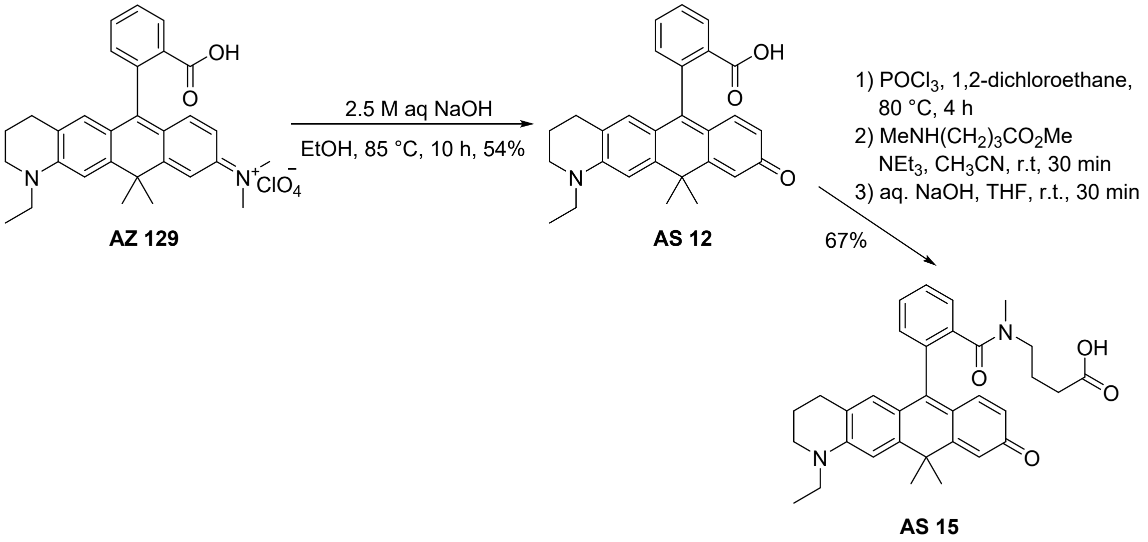
Figure S4. ^{13}C-NMR spectrum (125 MHz) of AS 14 in CD_3OD.

AS 14 LCMS (ESI$^+$): *m*/*z* calcd for $C_{31}H_{32}N_2O_4$ $[M + H]^+$: 497.2, found 497.4.

1.2. Synthesis of AS 15 via AS 12

1.2.1. 2-(1-Ethyl-11,11-dimethyl-9-oxo-1,2,3,4,9,11-hexahydronaphtho[2,3-*g*]quinolin-6-yl)benzoic acid (AS 12) synthesis

AZ 129 (0.30 g, 0.48 mmol) was dissolved in a 1:1 mixture of ethanol (35 mL) and 2.5 M aq. NaOH (35 mL, 87.5 mmol). The reaction mixture was stirred at 85 °C for 10 h (Scheme S2, conversion checked by HPLC). The color of the solution changed from blue to red after 2 h. After the completion of the reaction, the reaction mixture was cooled to 0 °C and acidified to pH 5.0 using TFA. The organic layer was separated using DCM, and the aqueous phase was extracted with DCM (3 × 30 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by reverse phase column chromatography (C18 modified SiO_2, 120 g; H_2O/CH_3CN/TFA 8/2/0.001). The red-colored fractions were collected and concentrated under reduced pressure, yielding AS 12 as a red solid (110 mg, 0.259 mmol, 54%).



Scheme S2: AS 15 synthesis.

AS 12 HPLC: CH_3CN/H_2O + 0.1 v/v% TFA = 30/70 to 95/5 in 15 min, detection at 550 nm, t_R = 5.12 min (>99%).

AS 12 ^{1}H-NMR (500 MHz, DMSO-d_6): δ = 1.10 (t, ^3J = 7 Hz, 3 H, 1-CH_2-$C\underline{H_3}$), 1.64 (s, 3 H, 11-CH_3), 1.73 (s, 3 H, 11-CH_3’), 1.75–1.80 (m, 2 H, 3-H), 2.36–2.47 (m, 2 H, 4-H), 3.23 (t, ^3J = 7 Hz, 2 H, 2-H), 3.44 (q, ^3J = 7 Hz, 2 H,1-CH_2), 6.13 (s, 1 H, 5-H), 6.44 (d, ^3J = 9 Hz, 1 H, 7-H), 6.59 (dd, ^3J = 8 Hz, ^4J = 2 Hz, 1 H, 8-H), 6.78 (s, 1 H, 12-H), 7.08 (d, ^3J = 8 Hz, 1 H, 6’-H), 7.09 (d,

^4J = 2 Hz, 1 H,10-H), 7.63 (ddd, ^3J = 7 Hz, ^4J = 2 Hz, 1 H, 4’-H), 7.70 (ddd, ^3J = 7 Hz, ^4J = 2 Hz, 1 H, 5’-H), 7.95 (d, ^3J = 7 Hz, 1 H, 3’-H) (Fig. S5).

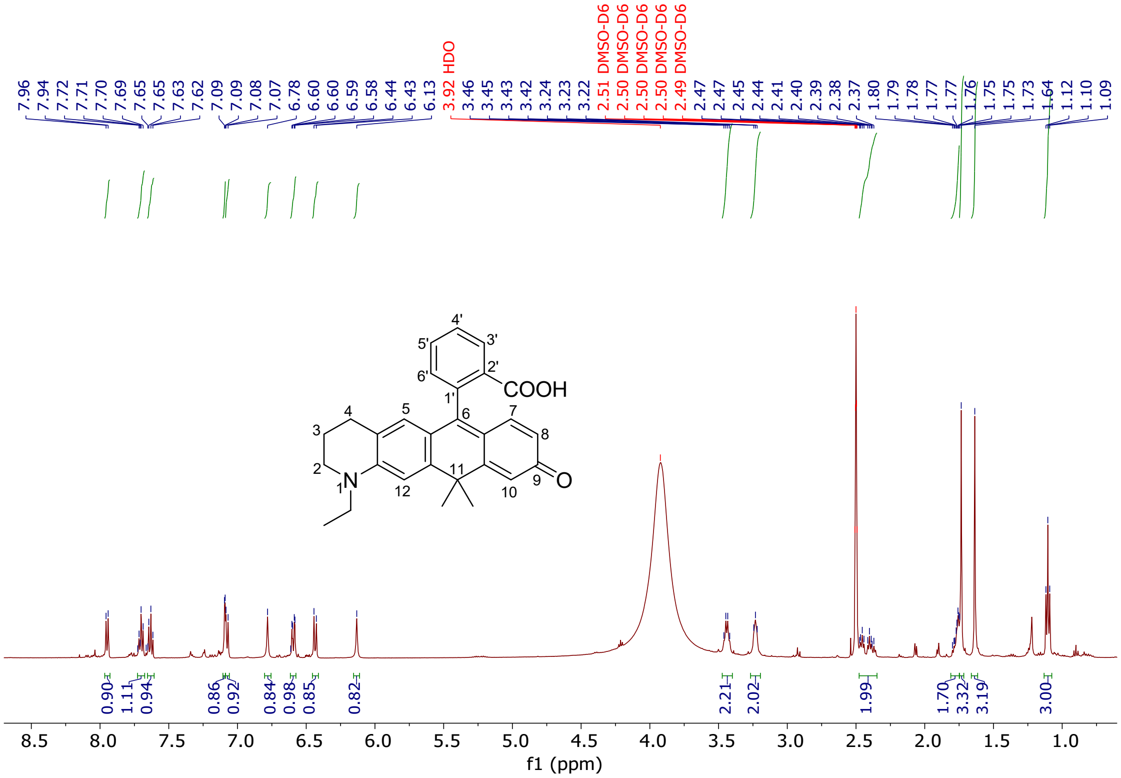


Figure S5. ^{1}H-NMR spectrum (500 MHz) of AS 12 in DMSO-d_6.

AS 12 ^{13}C-NMR (125 MHz, DMSO-d_6): δ = 10.4 (1-CH$_2$-$\underline{C}$H$_3$), 21.4 (C3), 27.1 (C4), 33.1 (11-CH$_3$’), 34.4 (11-CH$_3$), 37.7 (C11), 44.9 (1-CH$_2$), 48.0 (C2), 107.5 (C12), 112.8 (C10), 114.8 (C8), 117.3 (C5a), 121.9 (C4a), 122.1 (C6a), 124.2 (C6’), 125.0 (C3’), 126.2 (C2’), 127.6 (C5), 128.7 (C6), 129.1 (C7), 129.4 (C4’), 135.1 (C5’), 144.7 (C11a), 146.0 (C12a), 147.6 (C10a), 158.2 (C1’), 158.5 (C9), 169.8 (C2’-COOH) (Fig. S6).

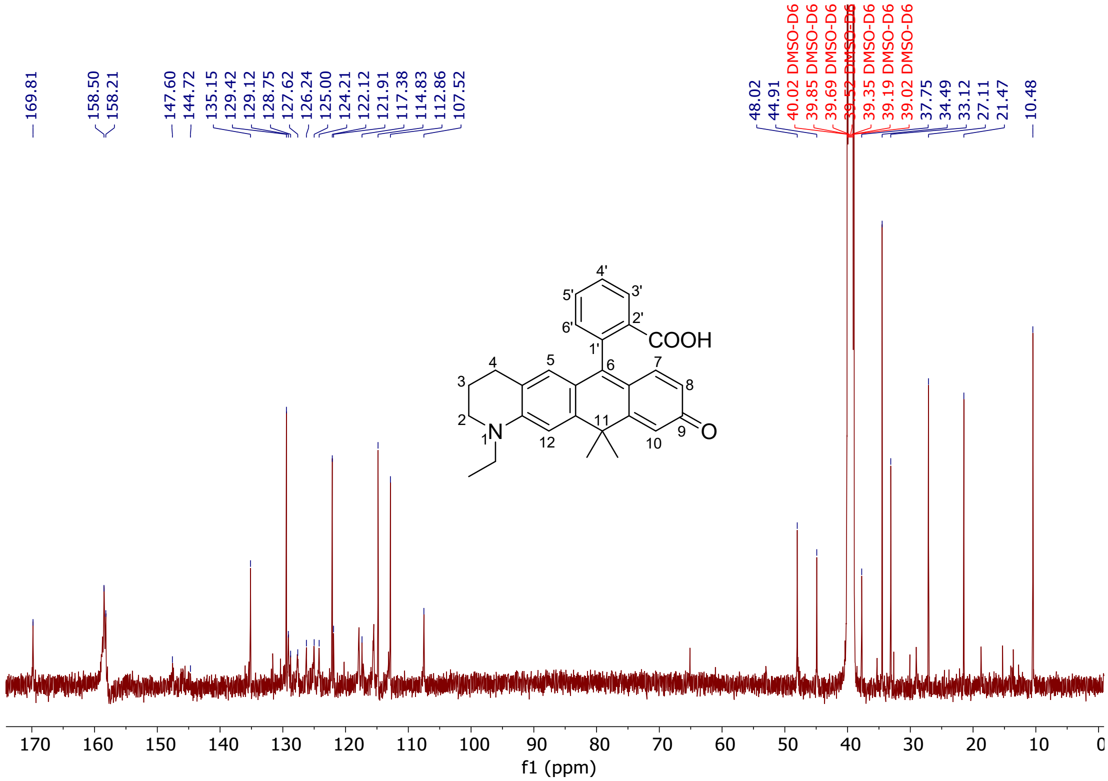


Figure S6. ^{13}C-NMR spectrum (500 MHz) of AS 12 in DMSO-d_6

AS 12 LCMS (ESI$^+$): *m*/*z* calcd for $C_{28}H_{27}NO_3$ $[M + H]^+$: 426.2, found 426.3.

1.2.2. 4-(2-(1-Ethyl-11,11-dimethyl-9-oxo-1,2,3,4,9,11-hexahydronaphtho[2,3-*g*] quinolin-6-yl)-*N*-methylbenzamido)butanoic acid (AS 15) synthesis

To a solution of AS 12 (80.0 mg, 148 μmol) in 1,2-dichloroethane (10 mL), $POCl_3$ (704 μL, 7.50 mmol) was added (Scheme S2). After stirring at 80 °C for 4 hours, all volatile materials were evaporated *in vacuo*, and the solid residue was dissolved in CH_3CN (5 mL). A solution of methyl-4-(methylamino) butanoate (97 mg, 0.74 mmol) in CH_3CN (5 mL) was added, followed by the addition of NEt_3 (842 μL, 6.00 mmol). After stirring at room temperature for 30 min, all volatile materials were removed *in vacuo*. The residue was redissolved in DCM (20 mL) and washed with a saturated aq. NH_4Cl. The organic layer was separated, dried with anhydrous Na_2SO_4, and evaporated.

For the hydrolysis of the ester group, the crude product was stirred for 5 h at room temperature in a mixture of H_2O (22 mL), THF (34 mL), and aq. NaOH (c = 1 M, 1.4 mL). Acetic acid (23 mL) was added, and the mixture was evaporated to dryness. Purification of the crude product by reverse phase column chromatography (C18 modified SiO_2, 40 g; H_2O/CH_3CN/TFA 8/2/0.001) yielded AS 15 (63 mg, 98.6 µmol, 67%) as a red solid.

AS 15 HPLC: CH_3CN/H_2O + 0.1 v/v% TFA = 15/85 to 95/5 in 15 min, detection at 550 nm, t_R = 6.56 min (>99%).

AS 15 ^{1}H-NMR (500 MHz, CD_3OD): δ = 1.41 (t, ^3J = 7 Hz, 3 H,1-CH_2-$C\underline{H_3}$), 1.43–1.50 (m, 2 H, CH_2'), 1.70 (s, 3 H, 11-CH_3), 1.80 (s, 3 H, 11-CH_3'), 1.83–1.90 (m, 2 H, CH_2''), 1.96–2.05 (m, 2 H, 3-H), 2.65–2.70 (m, 2 H, 4-H), 2.94 (s, 3 H, 2'CON($C\underline{H_3}$)), 3.17–3.38 (m, 2 H, CH_2), 3.76 (t, ^3J = 6.0 Hz, 2 H, 2-H), 3.83–3.98 (m, 2 H, 1-CH_2), 6.79 (dd, ^3J = 9.0 Hz, ^4J = 2.0 Hz, 1 H, 8-H), 6.97 (s, 1 H, 5-H), 7.13 (d, ^3J = 9.0 Hz, 1 H, 7-H), 7.28 (s, 1 H, 12-H), 7.30 (d, ^4J = 2.0 Hz, 1 H, 10-H), 7.43–7.47 (m, 1 H, 6'-H), 7.62–7.65 (m, 1 H, 3'-H), 7.69–7.73 (m, 2 H, 4'-H & 5'-H) (Fig. S7).

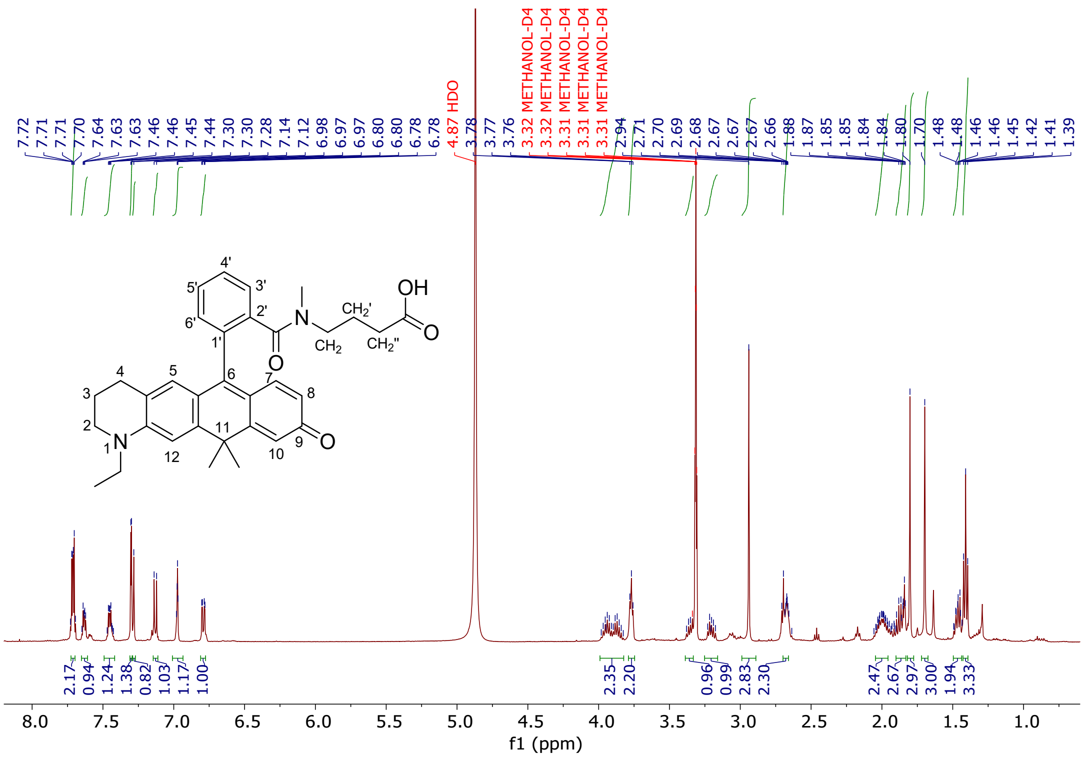


Figure S7. ^{1}H-NMR spectrum (500 MHz) of AS 15 in CD_3OD.

AS 15 ^{13}C-NMR (125 MHz, CD_3OD): δ = 12.3 (1-CH_2-$\underline{C}H_3$), 21.8 (C3), 23 (CH_2'), 27.6 (C4), 31.7 (CH_2''), 32.1 (11-CH_3'), 35.2 (11-CH_3), 37.9 (2'-CON($\underline{C}H_3$)), 42.5 (C11), 47.5 (CH_2), 49.1 (1-CH_2), 52.2 (C2), 113.4 (C12), 115.7 (C10), 116.5 (C8), 124.0 (C5a), 124.3 (C6a), 127.5 (C4a), 128.4 (C3'), 130.4 (C4' & C5'), 131.7 (C6'), 135.5 (C2'), 137.4 (C7), 137.4 (C1'), 137.4 (C5), 155.8 (C10a), 157.5 (C12a), 160.9 (C11a), 162.9 (C6), 166.3 (C9), 170.6 (C2'-$\underline{C}ONCH_3$), 176.2 (C2'-CON(CH_3)(CH_2)$_3$$\underline{C}$OOH) (Fig. S8).

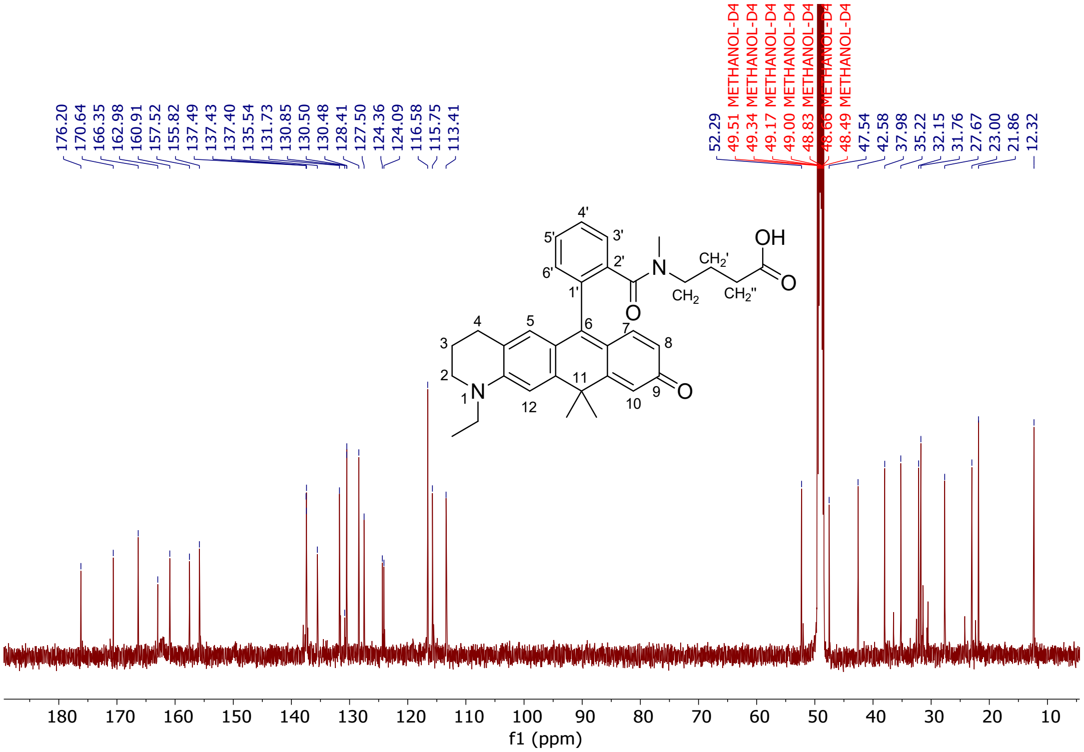


Figure S8. ^{13}C-NMR spectrum (125 MHz) of AS 15 in CD_3OD.

AS 15 LCMS (ESI$^+$): *m*/*z* calcd for $C_{33}H_{36}N_2O_4$ $[M + H]^+$: 525.2, found 525.4.

2. Photographs of spectroscopic setups

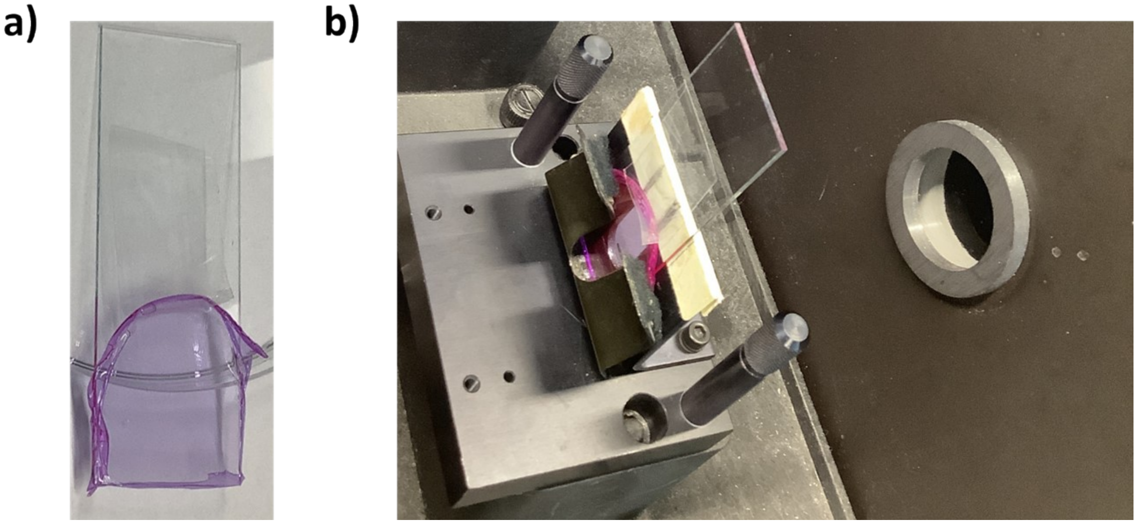


Figure S9. Photographs of the experimental setup for measuring p*K*$_a$. a) Modified CS film attached to a microscope slide, then b) placed in the UV-Vis sample holder.

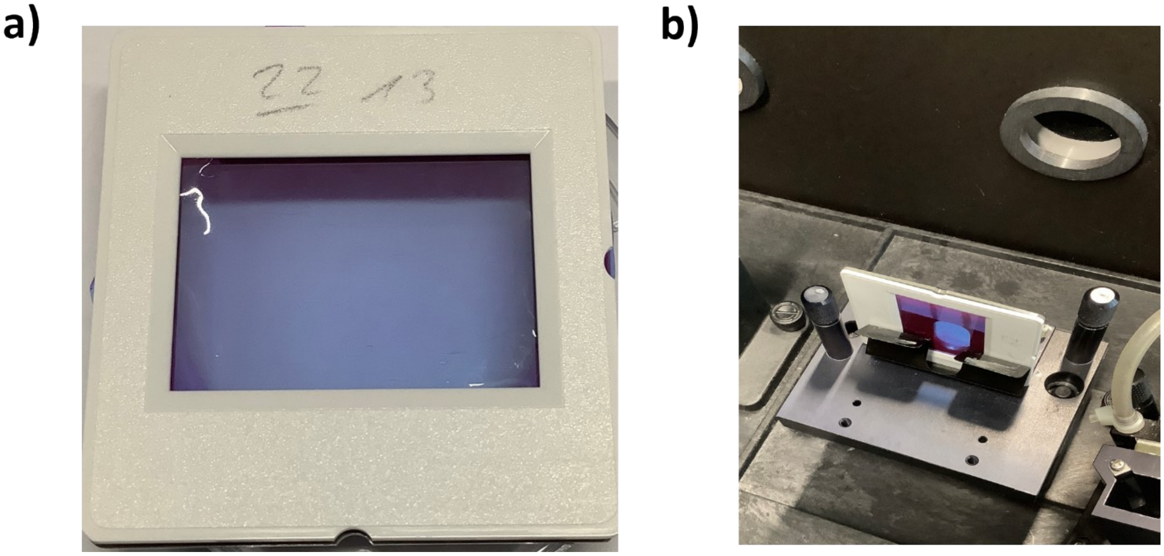


Figure S10. Photographs of the alternative experimental setup for measuring absorbance. a) Modified CS film is mounted on a 35 mm slide mount then b) placed in the UV-Vis sample holder.

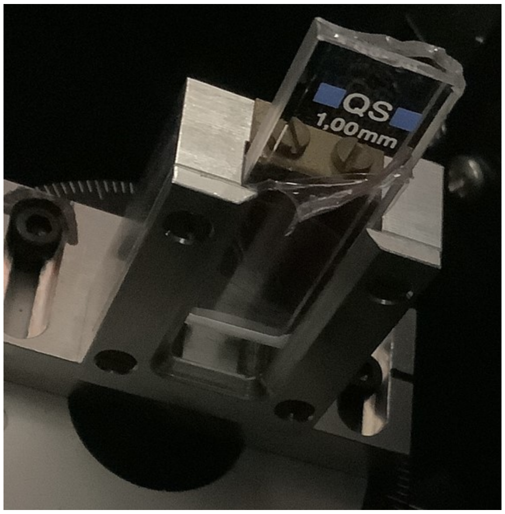


Figure S11. Photographs for the experimental setup to measure fluorescence emission spectra of drop-casted films.

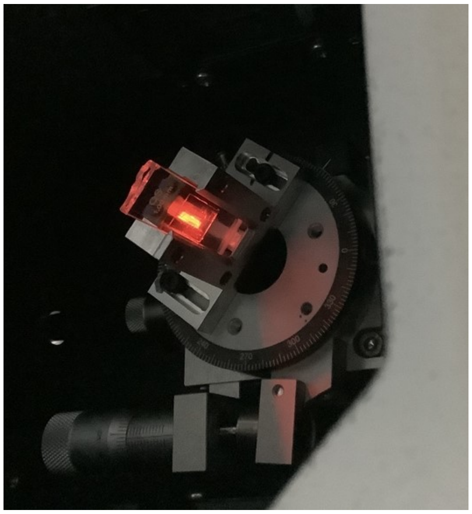


Figure S12. Photograph of the spin-coated coverslip attached to a 1 mm cuvette and placed in the fluorescence spectrometer sample holder.

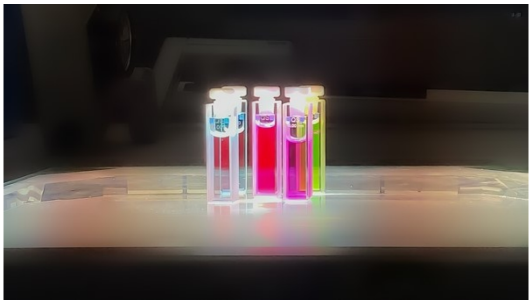


Figure S13. Photographs of the sample solutions were placed vertically in quartz cuvettes on top of the overhead projector for photostability measurements.

Figure S14. Photograph of CS films on a slide mount positioned horizontally on an overhead projector for photostability measurements.

3. Absorption and emission spectra of spin-coated films

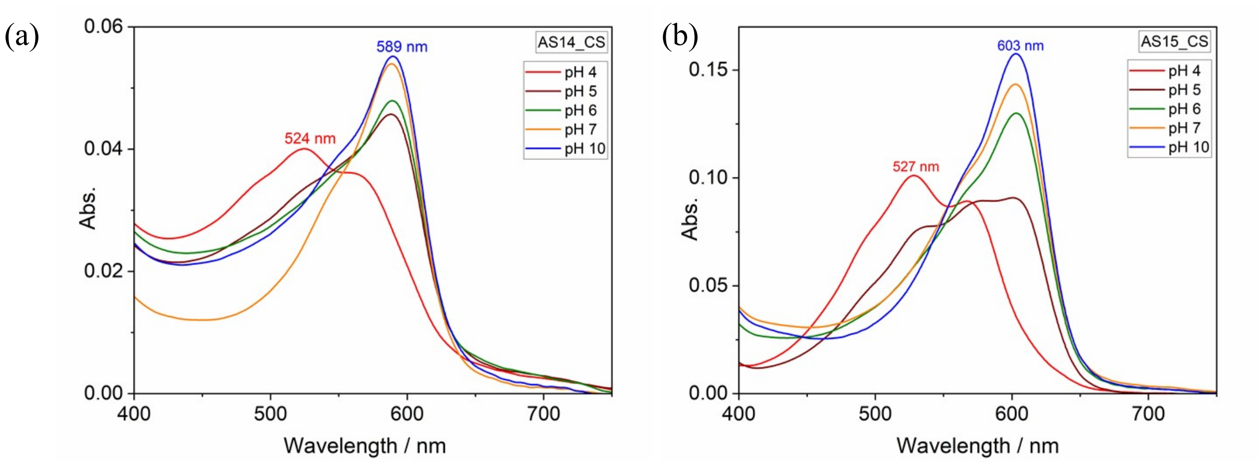


Figure S15. Absorption spectra of spin-coated a) AS14-CS and b) AS15-CS upon increasing pH.

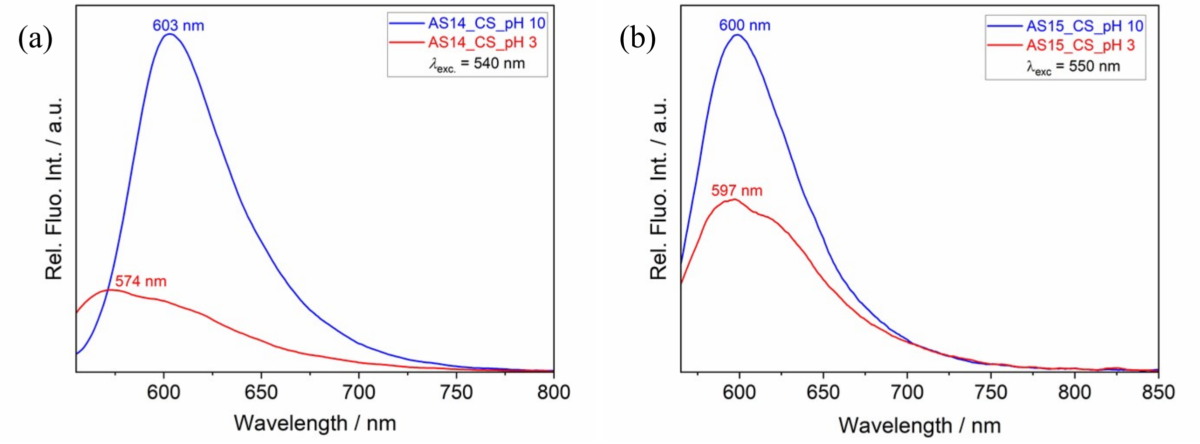


Figure S16. Fluorescence emission spectra of spin-coated a) AS14-CS and b) AS15-CS at pH 3 (red trace) and pH 10 (blue trace). λ_{ex} = 540 nm for AS14-CS and 550 nm for AS 15-CS.

4. FTIR spectroscopy band assignments

Table S1: Assignments of key bands observed in the ATR-FTIR spectra of non-modified CS, AS14-CS, and AS15-CS.

Assignment	**Band position (cm^{-1})**			
	Reference CS[2]	**Non-modified CS**	**AS 14-CS**	**AS 15-CS**
N-H (amide I)	1550-1565	1563	1574	1578 &1546
NH_2	1590-1610	1588	------	------
C=O (amide II)	1620-1665	1651	1650 &1630	1650

5. Stability of films in solution

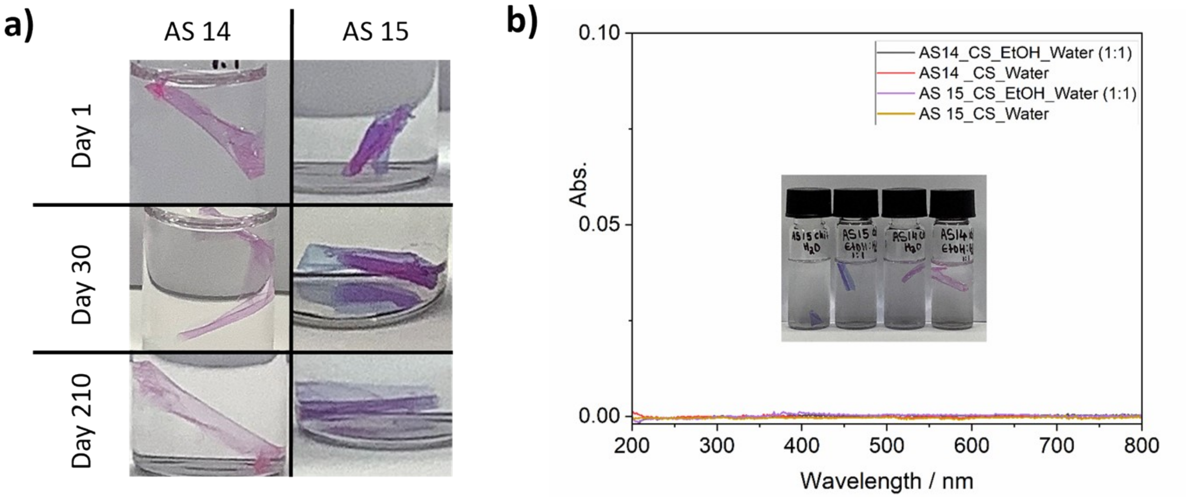


Figure S17. a) Photographs of AS 14-CS and AS 15-CS immersed in 50% ethanol after 1, 30 and 210 days. b) UV-Vis spectra of solutions obtained after immersing films in water or 50% ethanol for 24 h (measured in a 1 mm cuvette), showing the absence of dye release from AS14-CS and AS15-CS films. The inset shows a photograph of the films immersed in the corresponding solutions.

6. Film thickness determination

6.1. Drop-casted films

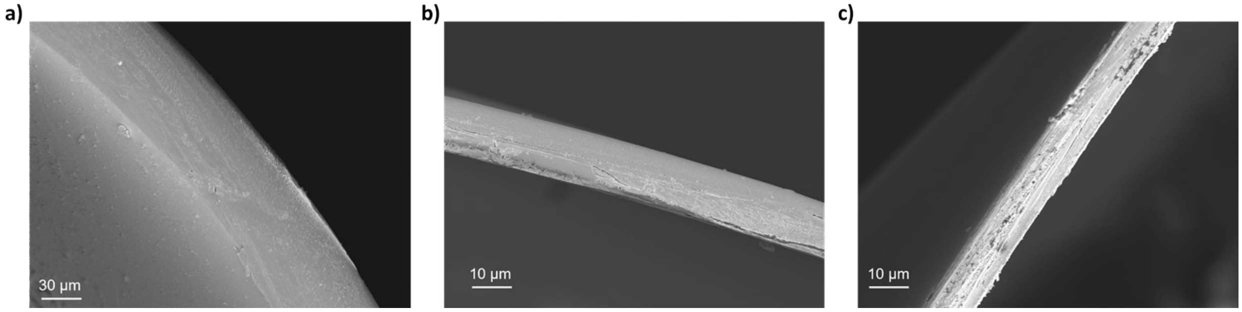


Figure S18. FESEM images of a) non-modified CS, b) AS 14-CS, and c) AS 15-CS.

6.2. Spin-coated films

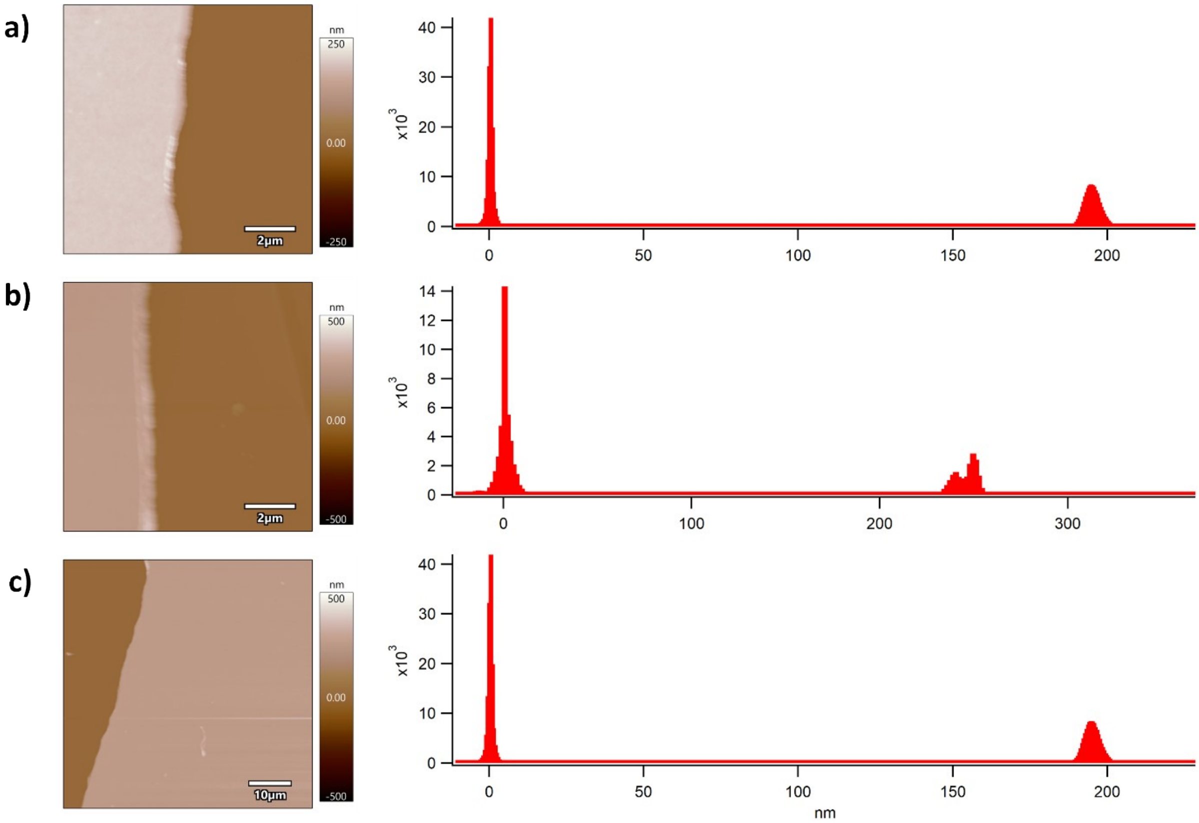


Figure S19. AFM micrographs of the scratch on spin-coated films and corresponding height distribution profiles (right) for a) non-modified CS, b) AS14-CS, and c) AS15-CS.

7. Solvent-dependent fluorescence lifetimes of AS 14 and AS 15

Table S2. Fluorescence lifetimes of the basic forms of AS 14 and AS 15, and the acidic form of AS 15 in polar protic solvents.(a)

Solvents	***τ* (ns); Basic form**		***τ* (ns); Acidic form**
	AS 14	**AS 15**	**AS 15**
Toluene	3.2	3.8	3.9
DCM	4.4	4.6	3.6
DMSO	4.2	4.4	2.8
2-Propanol	4.2	4.2	3.1
Ethanol	4.2	4.1	2.9
Buffer	2.3	2.7	1.6

(a)The data were analyzed using bi-exponential fitting, with χ^2 values between 0.900 and 1.200 and evenly distributed residuals taken as criteria for optimal fits. The lifetime of the dominant component was used to describe the decay behavior of the system, while the minor component (<5% contribution) was considered negligible. The acidic form of AS 14 was not measured due to its extremely weak emission and the very short lifetime observed for the analogous acidic form of the dye.

8. Photostability of AS 14 and AS 15 compared to known dyes

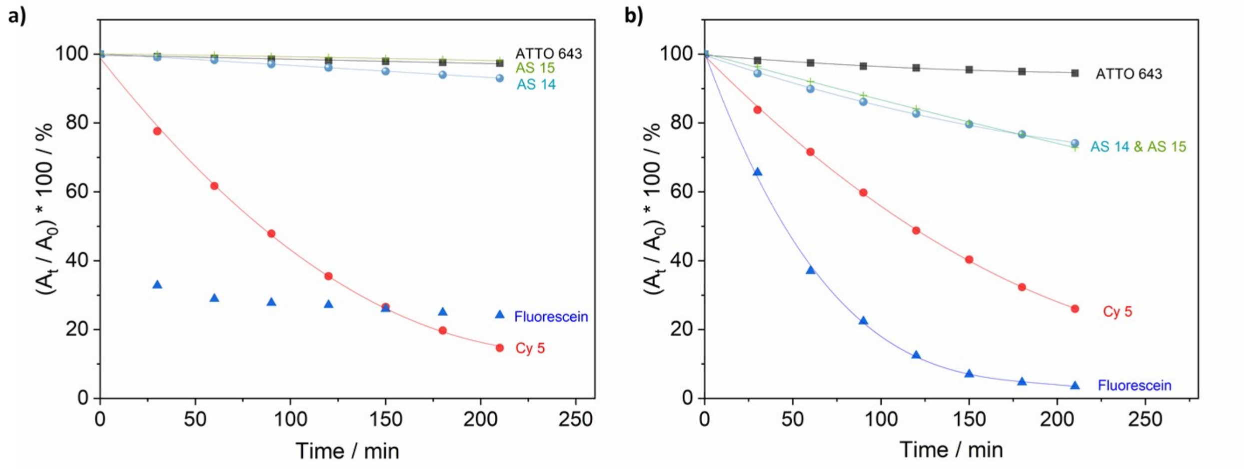


Figure S20: Photostability profile of the acidic form (a) and the basic form (b) in pH 3 and pH 10 buffer, respectively, over 3.5 hours of continuous irradiation (575 W mercury halide lamp).

9. pH-dependent absorption spectra of unmodified CS films

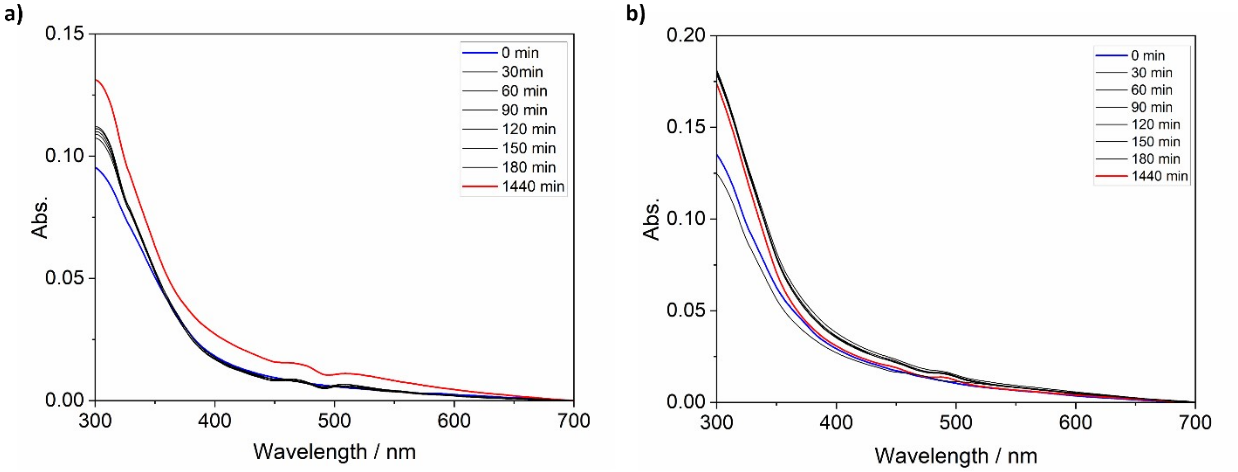


Figure S21. Absorption spectra of unmodified CS film washed with a) pH 4 buffer and b) pH 10 buffer.

10. References

(1) Syam, A.; Zilles, A.; Kemnitzer, N.; Arden-Jacob, J. Dyes with pH-dependent absorption and fluorescence. EP24200857.1, Sep 17, 2024.

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