Supporting Information

Water-Soluble Py-BIPS Spiropyrans as Photoswitches for Biological Applications

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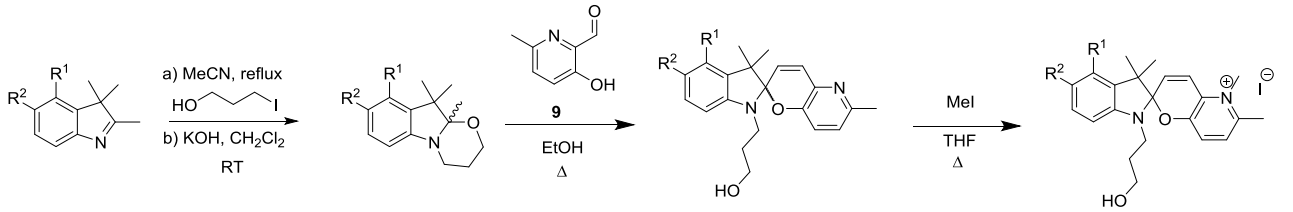
Syntheses

Compounds **5**,[S1] **6**,[S2] and **9**[S3] were prepared using reported procedures. NMR spectra for the characterization of the compounds were recorded on Bruker AVIII-HD 500 MHz instruments equipped with a N_2 cooled cryogenic probe head using $CDCl_3$ and d^6-DMSO as solvents. HRMS spectra were recorded using a Thermo Scientific MALDI LTQ Orbitrap.
For flash chromatography silica gel 60 by Macherey-Nagel was used. TLC analyses were performed on aluminum plates coated with silica gel 60 F 254 (Merck).

Supporting Scheme 1. Overview of the syntheses of the spiropyran derivatives **3** and **4**.

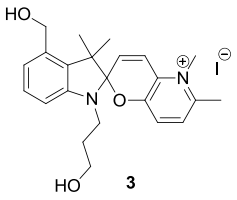


5: $R^1 = CH_2OH$, $R^2 = H$
6: $R^1 = H$, $R^2 = CH_2CH_2OH$

7: $R^1 = CH_2OH$, $R^2 = H$, (80 %)
8 $R^1 = H$, $R^2 = CH_2CH_2OH$, (99 %)

10: $R^1 = CH_2OH$, $R^2 = H$, (65 %)
11: $R^1 = H$, $R^2 = CH_2CH_2OH$, (63 %)

3: $R^1 = CH_2OH$, $R^2 = H$, (42 %)
4: $R^1 = H$, $R^2 = CH_2CH_2OH$, (63 %)



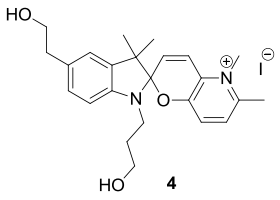
3

Synthesis of compound 3: Spiropyran **10** (0.1 g, 0.27 mmol) was dissolved in dry THF (5 mL). To the orange solution iodomethane (0.2 mL, 3.2 mmol) was added and the mixture was stirred in an autoclave under argon for 5 d. The resulting solid was filtered, washed with THF and dried to yield 42% of compound **3** (0.06 g, 0.12 mmol).

^{1}H-NMR (500 MHz, DMSO): δ = 7.85 (d, J = 8.8 Hz, 1H, H_{ar}), 7.73-7.70 (m, 2H, H_{ar}) 7.12 (t, J = 7.8 Hz, 1H, H_{ar}), 6.87 (d, J = 7.7 Hz, 1H, H_{ar}), 6.61-6.58 (m, 2H, -CH_2) 4.62 (d, J = 13.2 Hz, 1H, H_{ar}), 4.51 (d, J = 13.2 Hz, 1H), 4.15 (s, 3H, R_2N^+-CH_3), 3.43-3.41 (m, 2H, -CH_2), 3.32-3.26 (m, 1H, -CH_2), 3.20-3.15 (m, 1H, -CH_2), 2.69 (s, 3H, Ar-CH_3), 1.79-1.71 (m, 1H, -CH_2), 1.66-1.59 (m, 1H, -CH_2), 1.33 (s, 3H, -CH_3), 1.21 (s, 3H, -CH_3) ppm.

^{13}C-NMR (125.8 MHz, DMSO): δ = 151.5, 148.0, 147.3, 138.3, 134.3, 131.7, 131.0, 130.7, 129.0, 128.0, 123.0, 119.8, 107.3, 106.4, 67.5, 60.6, 58.7, 54.8, 31.9, 25.6, 23.9, 21.0, 20.4 ppm.

MALDI-HRMS: *m*/z calcd. for $C_{23}H_{29}N_2O_3$ $[M]^+$ 381.21727, found 381.21736 (Δm 0.00009, error 0.24 ppm).



4

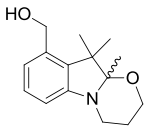
Synthesis of compound 4: Spiropyran **11** (0.1 g, 0.26 mmol) was dissolved in dry THF (5 mL). To the orange solution iodomethane (0.2 mL, 3.2 mmol) was added and the mixture was stirred in an autoclave under argon for 5 d. The resulting solid was filtered, washed with THF and dried to yield in 63% of compound **4** (0.09 g, 0.17 mmol).

^{1}H-NMR (500 MHz, DMSO): δ = 7.89 (d, J = 8.8 Hz, 1H, H_{ar}), 7.72-7.67 (m, 2H, H_{ar}), 6.99-6.96 (m, 2H, H_{ar}), 6.60-6.57 (m, 2H, H_{ar}), 4.58 (s, 1H, -OH), 4.47 (s, 1H, -OH), 4.14 (s, 3H, R_2N^+-CH_3), 3.58 (t, J = 7.0 Hz, 2H, -CH_2), 3.42 (t, 2H, -CH_2), 3.30-3.24 (m, 1H, -

CH_2), 3.18-3.12 (m, 1H, -CH_2), 2.69 (s, 3H, Ar-CH_3), 2.67 (t, J = 7.1 Hz, 2H, -CH_2), 1.79-1.71 (m, 1H, -CH_2), 1.66-1.59 (m, 1H, -CH_2), 1.24 (s, 3H, -CH_3), 1.12 (s, 3H, -CH_3) ppm.

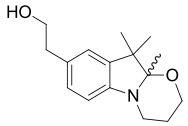
^{13}C-NMR (125.8 MHz, DMSO): δ = 151.3, 148.1, 145.2, 135.5, 134.5, 132.1, 131.0, 130.8, 129.0, 128.5, 122.8, 122.4, 106.9, 106.8, 67.5, 63.1, 58.7, 53.7, 39.2, 31.9, 26.0, 25.6, 21.0, 20.2 ppm.

MALDI-HRMS: *m/z* calcd. for $C_{24}H_{31}N_2O_3$ $[M]^+$ 395.23292, found 395.23308 (Δm 0.00016, error 0.41 ppm).



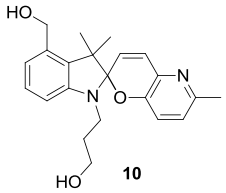
7

Synthesis of compound 7: Compound **5** (1.4 g, 7.4 mmol) was dissolved in dry acetonitrile (50 mL). To the orange solution 3-iodo-propan-1-ol (1.5 mL, 15.9 mmol) was added and the red mixture was refluxed for 18 h. The acetonitrile was evaporated and the resulting purple oil was washed with petrol ether and diethyl ether. After drying under vacuum a dark purple foam was obtained which was suspended in aqueous KOH (50 mL, 1 M) and stirred for 2 h at r.t. Dichloromethane (30 mL) was added and the solution was stirred for additional 30 min. The aqueous layer was separated and extracted two times with dichloromethane. The combined organic layers were washed with brine and H_2O and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue (80%, 1.47 g, 5.9 mmol) was used for the following step without further purification.



8

Synthesis of compound 8: Compound **6** (8 g, 39.4 mmol) was dissolved in dry acetonitrile (35 mL). To the orange solution 3-iodo-propan-1-ol (4.49 mL, 46.8 mmol) was added and the red mixture was refluxed for 18 h. The acetonitrile was evaporated and the resulting purple oil was washed with petrol ether and diethyl ether. After drying under vacuum a red oil was obtained which was suspended in aqueous KOH (100 mL, 1 M) and stirred for 2 h at r.t. Dichloromethane (30 mL) was added and the solution was stirred for additional 30 min. The aqueous layer was separated and extracted two times with dichloromethane. The combined organic layers were washed with brine and H_2O and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue (99%, 10.3 g, 39.3 mmol) was used for the following step without further purification.



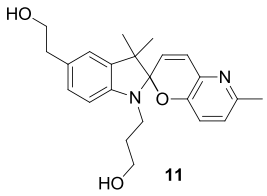
10

Synthesis of compound 10: 3-hydroxy-6-methyl-2-pyridinecarboxy-aldehyde **9** (800 mg, 5.8 mmol) and indoline **7** (645 mg, 2.6 mmol) were dissolved in dry EtOH (40 mL) and the dark-violet mixture was refluxed for 16 h. The ethanol was evaporated to a small volume and 10 mL of cold water were added. The mixture was stirred in an ice bath overnight. The resulting precipitate was filtered, washed with water and dried under vacuum to yield compound **10** (65%, 1.4 g, 3.8 mmol) as a tan solid.

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 7.18 (t, J = 7.8 Hz, 1H, H_{ar}), 7.05 (d, J = 10.6 Hz, 1H, H_{ar}), 6.89-6.87 (m, 3H, H_{ar}), 6.61 (d, J = 7.8 Hz, 1H, H_{ar}), 5.93 (d, J = 10.6 Hz, 1H, H_{ar}), 4.8 (q, J = 12.5 Hz, 2H, -CH_2), 3.69 (t, J = 6.1 Hz, 2H, -CH_2), 3.41-3.35 (m, 1H, -CH_2), 3.26-3.20 (m, 1H, -CH_2), 2.45 (s, 3H, Ar-CH_3), 1.96-1.88 (m, 1H, -CH_2), 1.83-1.75 (m, 1H, -CH_2), 1.42 (s, 3H, -CH_3), 1.26 (s, 3H, -CH_3) ppm.

^{13}C-NMR (125.8 MHz, $CDCl_3$): δ = 149.6, 149.1, 148.1, 137.3, 136.4, 132.6, 131.8, 128.1, 124.0, 123.4, 122.4, 119.6, 106.8, 105.4, 62.4, 60.9, 53.5, 41.1, 31.7, 24.2, 23.4, 20.8 ppm.

MALDI-HRMS: *m/z* calcd. for $C_{22}H_{26}N_2O_3$ $[M+H]^+$ 367.20162, found 367.20221 (Δm 0.0006, error 1.61 ppm).



11

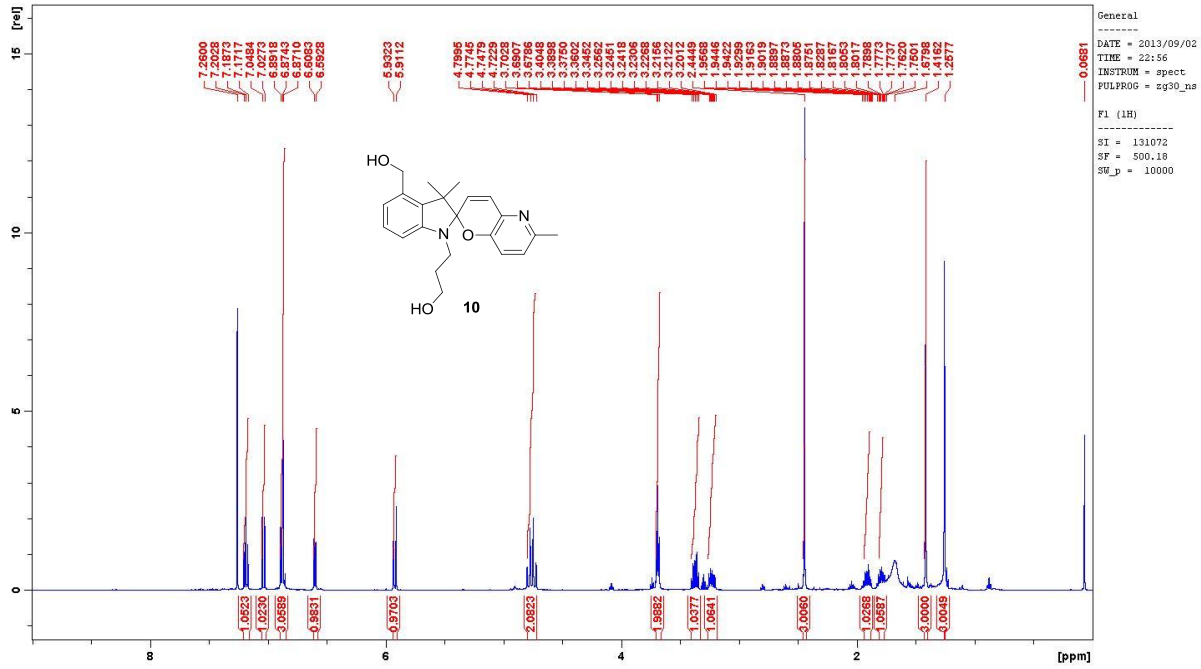
Synthesis of compound 11: 3-hydroxy-6-methyl-2-pyridinecarboxy-aldehyde **9** (400 mg, 2.9 mmol) and indoline **8** (1.29 g, 5.2 mmol) were dissolved in dry EtOH (80 mL) and the dark-violet mixture was refluxed for 16 h. The ethanol was evaporated and the residue purified via column chromatography with ethyl acetate to yield compound **11** (63%, 700 mg, 1.84 mmol) as a blue foam.

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 7.02 (m, 2H, H_{ar}), 6.93 (d, J = 1.6 Hz, 1H, H_{ar}), 6.90 (q, J = 8.4 Hz, 2H, H_{ar}), 6.57 (d, J = 7.9 Hz, 1H, H_{ar}), 5.94 (d, J = 10.5 Hz, 1H, H_{ar}), 3.85 (t, J = 6.6 Hz, 2H, -CH_2), 3.70 (t, J = 6.1 Hz, 2H, -CH_2), 3.39-3.33 (m, 1H, -CH_2), 3.23-3.18 (m, 1H, -CH_2), 2.83 (t, J = 6.6 Hz, 2H, -CH_2), 2.44 (s, 3H, Ar-CH_3), 1.95-1.87 (m, 1H, -CH_2), 1.83-1.75 (m, 1H, -CH_2), 1.27 (s, 3H, -CH_3), 1.16 (s, 3H, -CH_3) ppm.

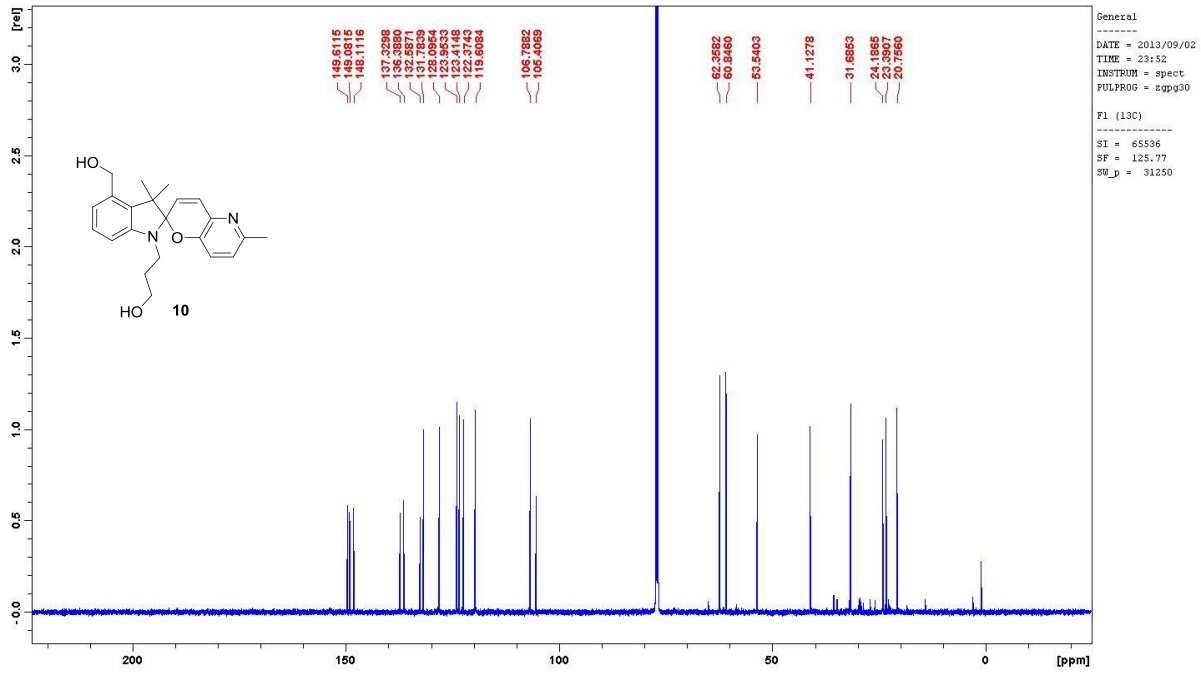
^{13}C-NMR (125.8 MHz, $CDCl_3$): δ = 149.6, 148.9, 146.1, 137.5, 136.9, 131.1, 129.1, 128.1, 124.1, 123.9, 122.5, 106.7, 105.3, 63.9, 60.7, 52.4, 41.0, 38.8, 31.8, 25.9, 23.3, 20.1 ppm.

MALDI-HRMS: *m/z* calcd. for $C_{23}H_{28}N_2O_3$ $[M+H]^+$ 381.21727, found 381.21728 (Δm 0.00001, error 0.03 ppm).

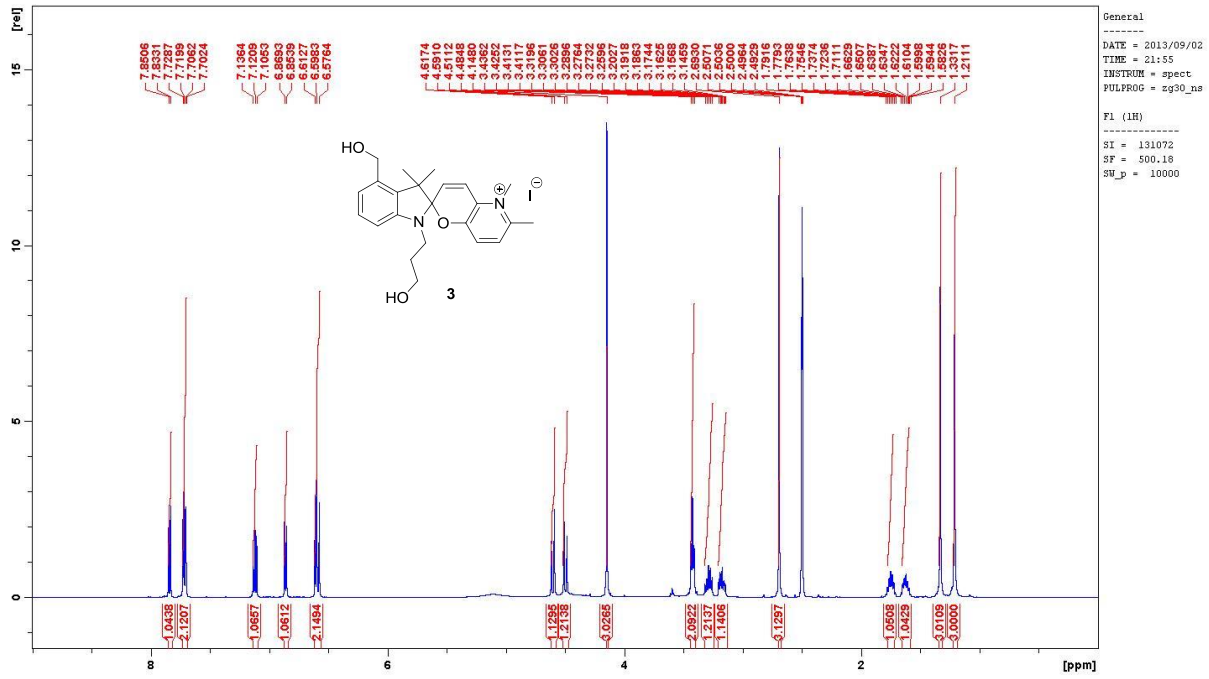
NMR spectra



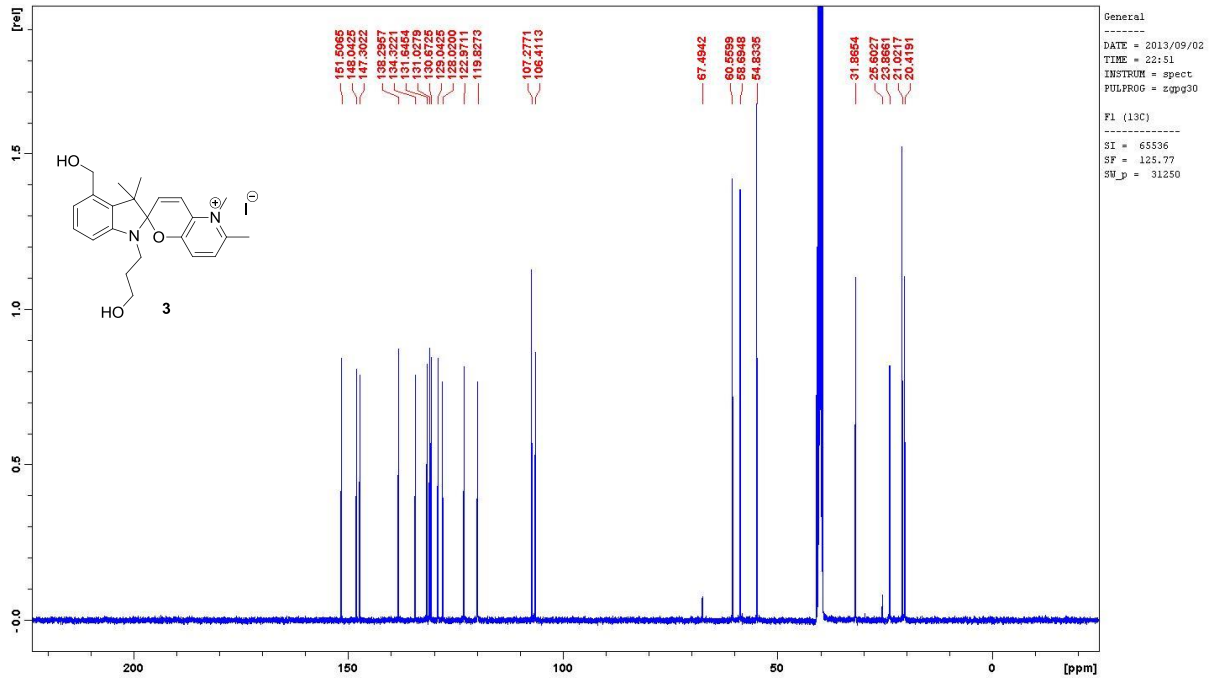
^{1}H-NMR of compound **10**



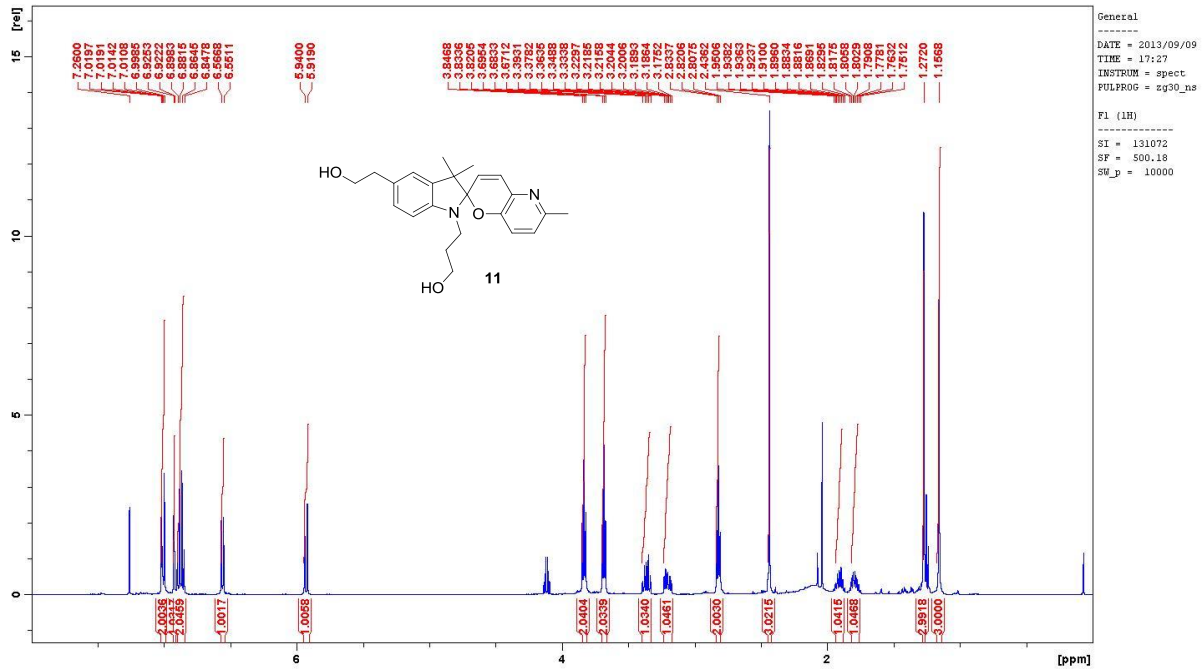
^{13}C-NMR of compound **10**



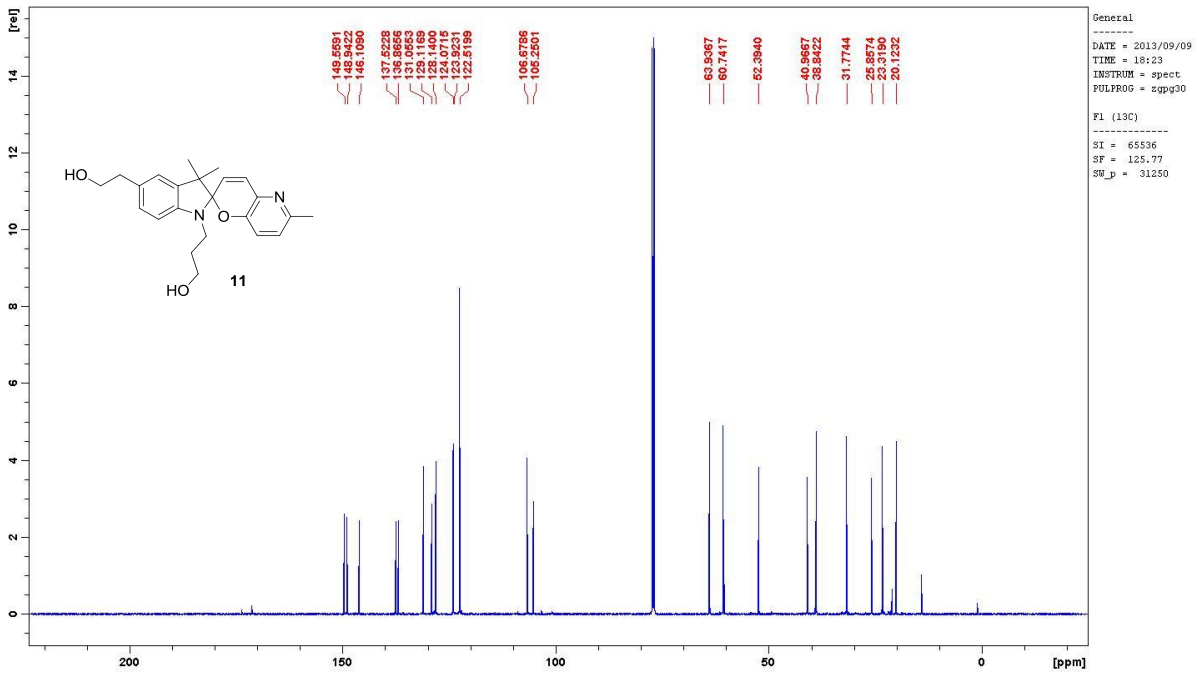
^{1}H-NMR of compound **3**



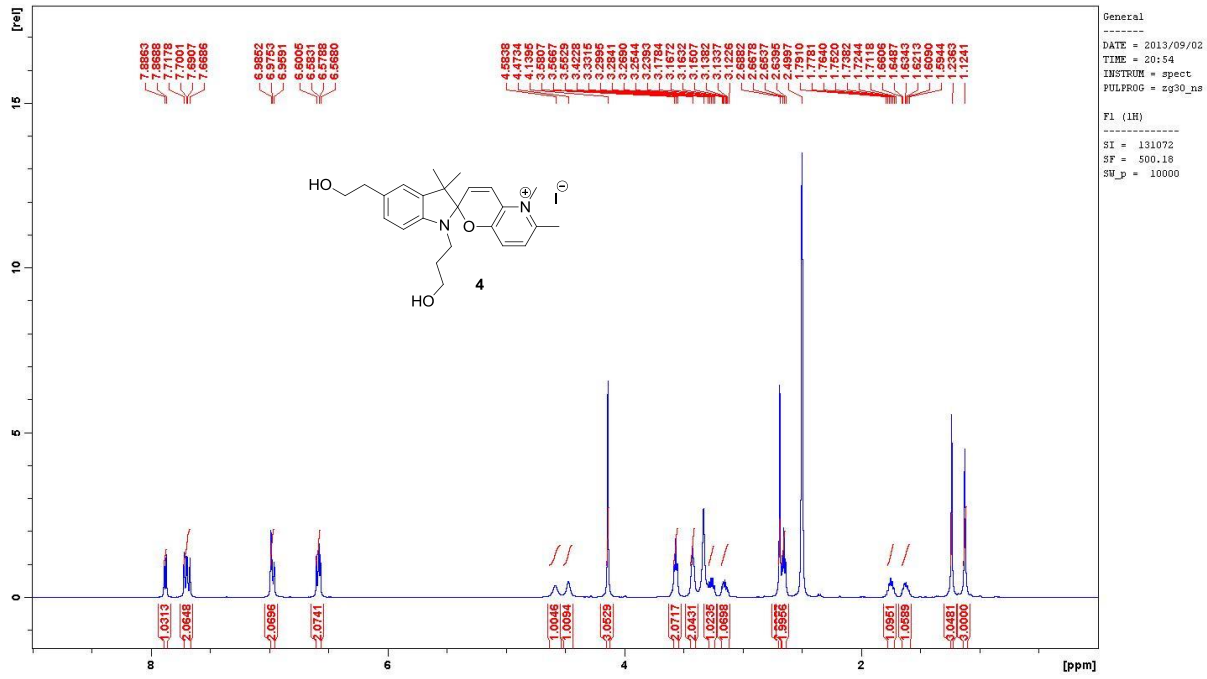
^{13}C-NMR of compound **3**



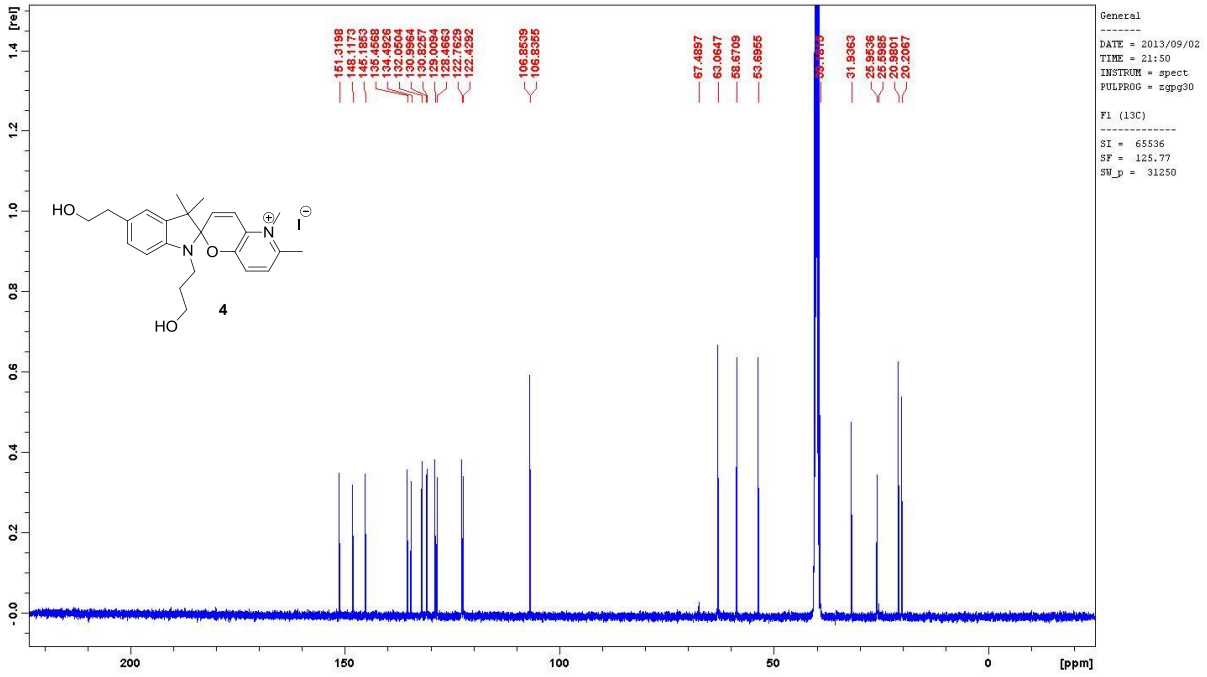
^{1}H-NMR of compound **11**



^{13}C-NMR of compound **11**



^{1}H-NMR of compound **4**



^{13}C-NMR of compound **4**

Data of optical spectroscopy

Absorption spectra were recorded using a Specord S100 UV/Vis spectrometer. For the quantum efficiency measurements the sample volume of 2 mL was stirred continuously to guarantee a homogeneous sample distribution. For the conversion from spiropyran to merocyanine a 365 nm LED (M365L2, Thorlabs) and for the reverse photoreaction a 530 nm LED (M530L2, Thorlabs) was used.

The photoswitching cycles were recorded using an Ocean Optics USB4000 spectrometer with a DH-mini UV-VIS-NIR-light source. For the conversion same LEDs as above were used.

HPLC studies for separation and determination of the UV-vis spectra of the two photoisomers were performed on a Agilent Technologies 1260 Infinity HPLC system with a MultoKrom 100-5 C4 (2 cm) precolumn (eluent: TEAA buffer pH 7.4 / MeCN, 90:10, flow 1 mL/min) and a diode array detector. The concentration of the samples was 1 mM. The UV irradiation at λ_{max}= 365 nm of the sample for this study was performed in a custom build irradiation setup containing three LEDs with 100 mW power each (Nichia NCCU033(T)).

$$\Phi = \frac{m \cdot V \cdot N_A \cdot h \cdot c}{P_0 \cdot \lambda \cdot (1 - 10^{-A_0}) \cdot \varepsilon_{prod} \cdot d}$$

Supporting Table 1. Calculation of quantum efficiency of ring opening. m = slope of the linear fit (time-dependent absorbance change during illumination), V = sample volume (= $(2 \cdot 10^{-3} \pm 2 \cdot 10^{-6})$ L), $N_A \cdot h \cdot c$ = constants, P_0 = irradiation intensity (= $(3.85 \cdot 10^{-3} \pm 4 \cdot 10^{-5})$ W), λ = excitation wavelength (= $3.65 \cdot 10^{-7}$ m), A_0 = absorbance at excitation wavelength, ε_{MC} = extinction coefficient of merocyanine at 520 nm, d = cuvette thickness (= 1 cm).

	m ± Δm $10^{-3} \cdot s^{-1}$	**A_0**	**$\varepsilon_{MC} \pm \Delta\varepsilon_{MC}$** $10^4 \cdot (M \cdot cm)^{-1}$	**$\Phi_o \pm \Delta\Phi_o$** %
2	1.44 ± 0.03	0.834	1.82 ± 0.04	1.6 ± 0.1
3	3.69 ± 0.08	0.543	2.00 ± 0.07	4.4 ± 0.2
4	3.61 ± 0.13	0.686	1.77 ± 0.03	4.4 ± 0.2

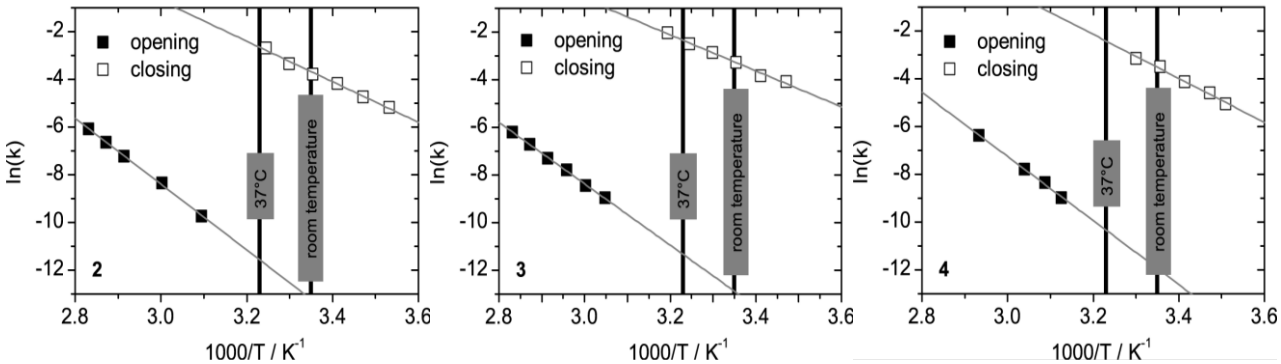
Supporting Table 2. Calculation of quantum efficiency of ring closing. m = slope of the linear fit (time-dependent absorbance change during illumination), V = sample volume (= $(2 \cdot 10^{-3} \pm 2 \cdot 10^{-6})$ L), $N_A \cdot h \cdot c$ = constants, P_0 = irradiation intensity (= $(1.10 \cdot 10^{-3} \pm 4 \cdot 10^{-5})$ W), λ = excitation wavelength (= $5.30 \cdot 10^{-7}$ m), A_0 = absorbance at excitation wavelength, ε_{SP} = extinction coefficient of spiropyran at 350 nm, d = cuvette thickness (= 1 cm).

	m ± Δm $10^{-3} \cdot s^{-1}$	**A_0**	**$\varepsilon_{SP} \pm \Delta\varepsilon_{SP}$** $10^3 \cdot (M \cdot cm)^{-1}$	**$\Phi_c \pm \Delta\Phi_c$** %
2	1.26 ± 0.05	0.327	9.8 ± 0.2	10.0 ± 0.6
3	7.90 ± 0.04	0.124	9.8 ± 0.1	13.3 ± 0.9
4	1.26 ± 0.05	0.376	9.7 ± 0.5	9.2 ± 0.7

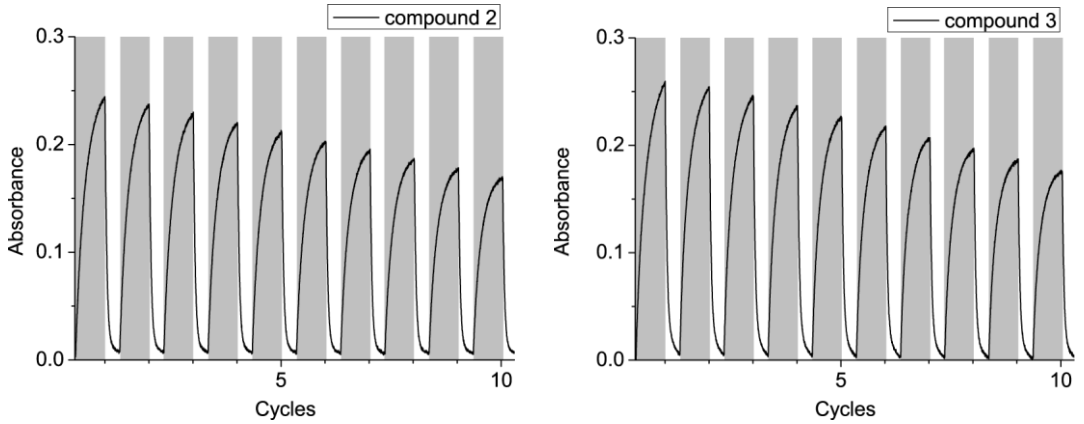
Supporting Table 3. Extinction coefficients of spiropyran at 350 nm and merocyanine[a)] at 520 nm of compounds **2**-**4** in aqueous solution.

	$\varepsilon_{SP} \pm \Delta\varepsilon_{SP}$ $(M \cdot cm)^{-1}$ 350 nm	**ε_{MC}[a)] $\pm \Delta\varepsilon_{MC}$** $(M \cdot cm)^{-1}$ 520 nm
2	9800 ± 200	18200 ± 400
3	9830 ± 130	20000 ± 700
4	9700 ± 500	17700 ± 300

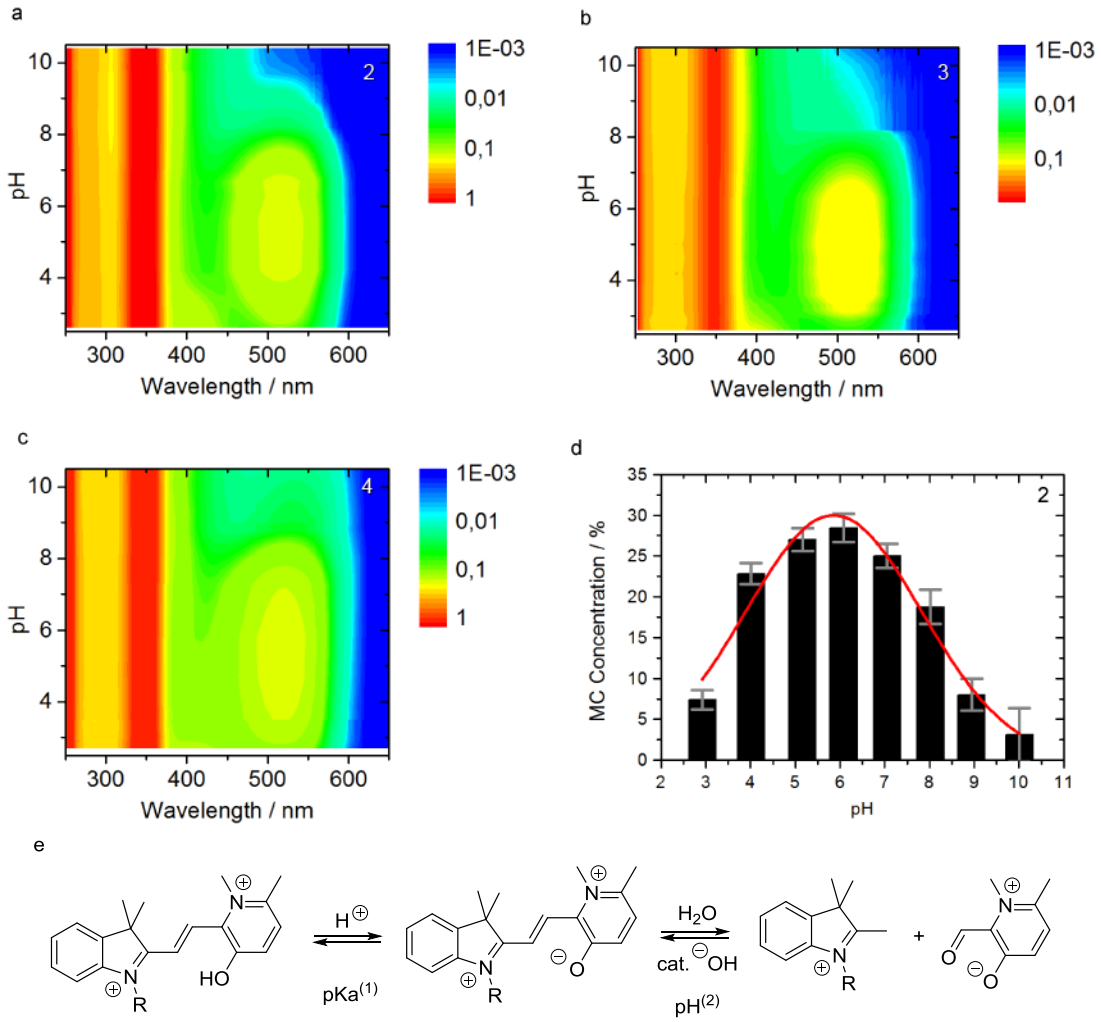
[a)] Extinction coefficients for merocyanine were determined in PBS/10% MeCN, on the assumption that no competition reaction to the ring opening reaction occurs, by calculating composition of spectrum of PSS by matching pure spectra of SP and MC from HPLC, which were recorded with addition of 10% MeCN.



Supporting Figure 1. Arrhenius graphs of thermal opening and closing reaction of compounds **2**, **3** and **4**.



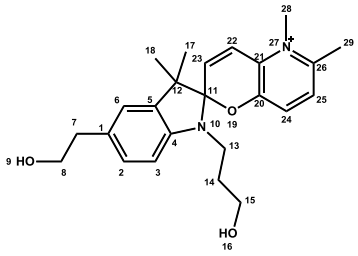
Supporting Figure 2. Absorbance changes of compound **2** and **3** (0.1 mM) at 531 nm upon alternating irradiation with UV (365 nm, grey areas) and visible light (530 nm, white areas).



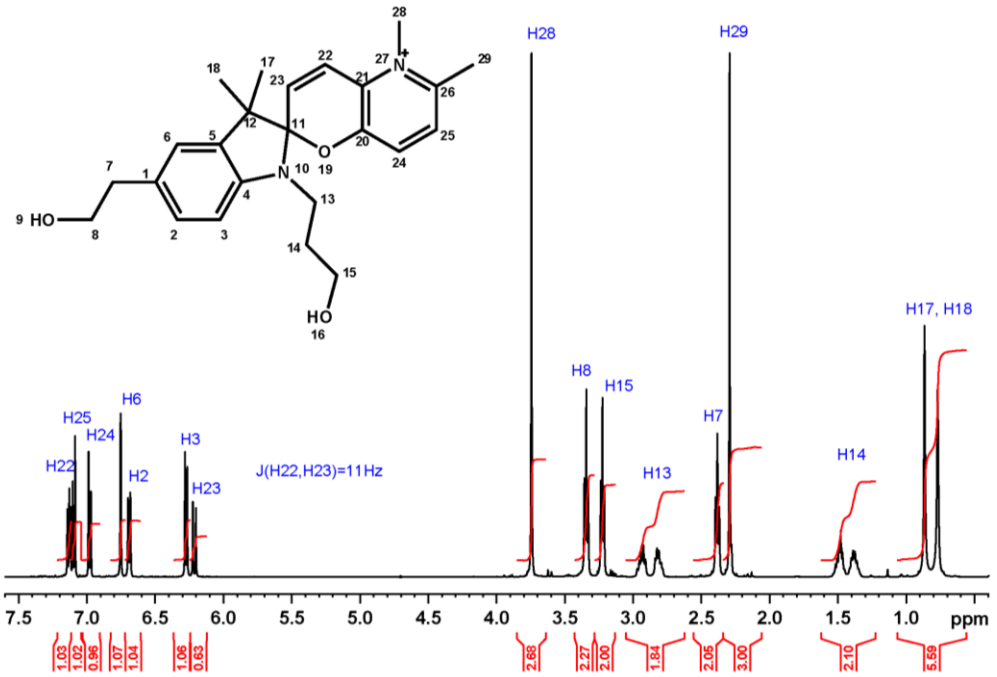
Supporting Figure 3. a-c) UV/vis absorbance spectra of compounds **2-4** at various pH values. d) Spectroscopically determined amount of unprotonated MC state of compound **2** at various pH values. e) Suggestion for the interpretation of the underlying processes.

NMR Studies

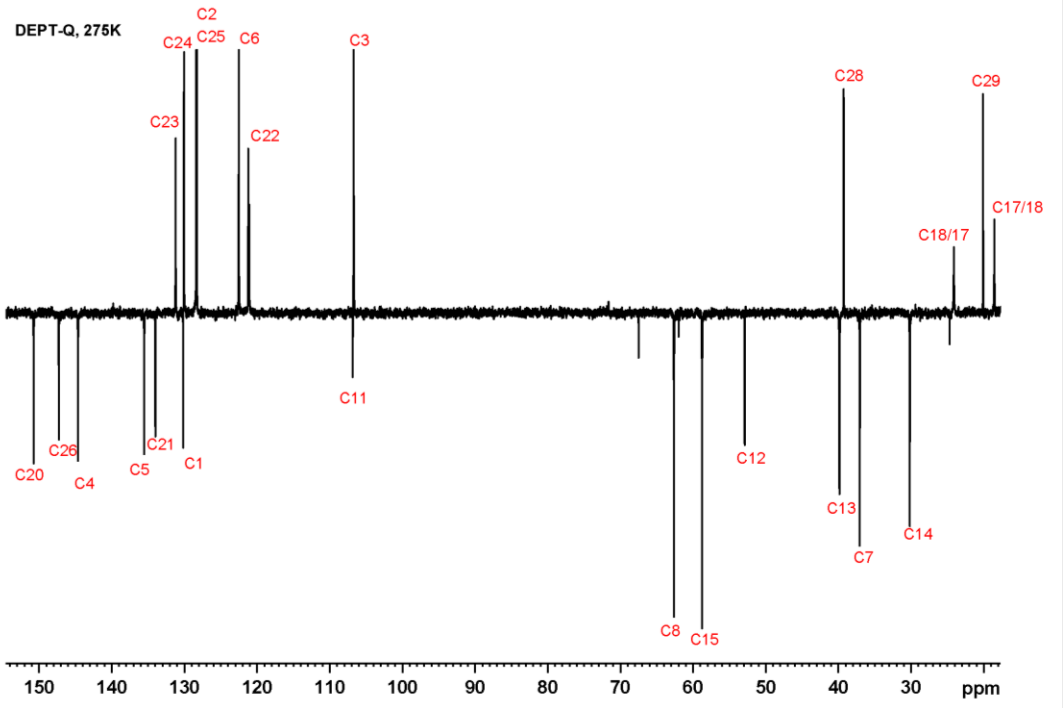
Assignment table



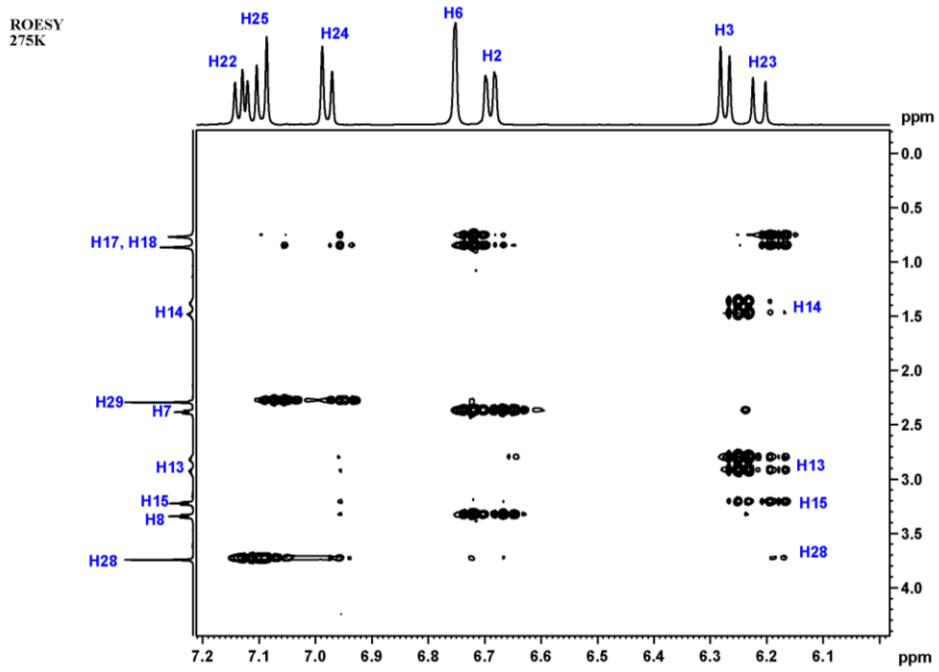
Position	^{1}H [ppm]	^{13}C [ppm]	COSY	ROESY	HMBC (H→C; H→N)
1	-	130.17	-	-	H3, H7, H8
2	6.65	128.36	H3	H7, H8	H6, H7
3	6.25	106.85	H2	H13, H14, H15	H2, H6
4	-	144.64	-	-	H2, H6, H13
5	-	135.54	-	-	H3, H17/18
6	6.70	122.52	-	H7, H8, H17, H18	H2, H3, H7
7	2.35	37.07	H8	H2, H6, H8	H2, H6, H8
8	3.30	62.64	H7	H2, H6, H7	(H6), H7
11	-	106.71	-	-	H13, H17/18, H22, H23
12	-	52.91	-	-	(H3, H12, H23), H6, H17, H18
13	2.90 | 2.80	39.28	-	H3,(H23)	H14, H15
14	1.50 | 1.40	30.19	H15	H3, H15, (H23)	H13, H15
15	3.20	58.78	H14	H3, H14, H23	H13, H14
17, 18	1.10 | 1.00	24.12 | 18.56	-	H6, H23	H17/18
20	-	150.75	-	-	H24, H25
21	-	133.99	-	-	H23, H24, H25, H28
22	7.10	121.21	H23	H23, H28	no HMBC peak
23	6.20	131.21	H22	(H14), H15, H17/18, H22	H22
24	6.95	130.06	H25	H29	H25
25	7.05	128.30	H24	H29	H29
26	-	147.29	-	-	H24, H25, H28, H29
28	3.75	39.28	-	H22	(H25), H29
29	2.25	20.10	-	H24, H25	H25
10	-	101.16	-	-	H3, H23
27	-	189.70	-	-	H25, H28, H29



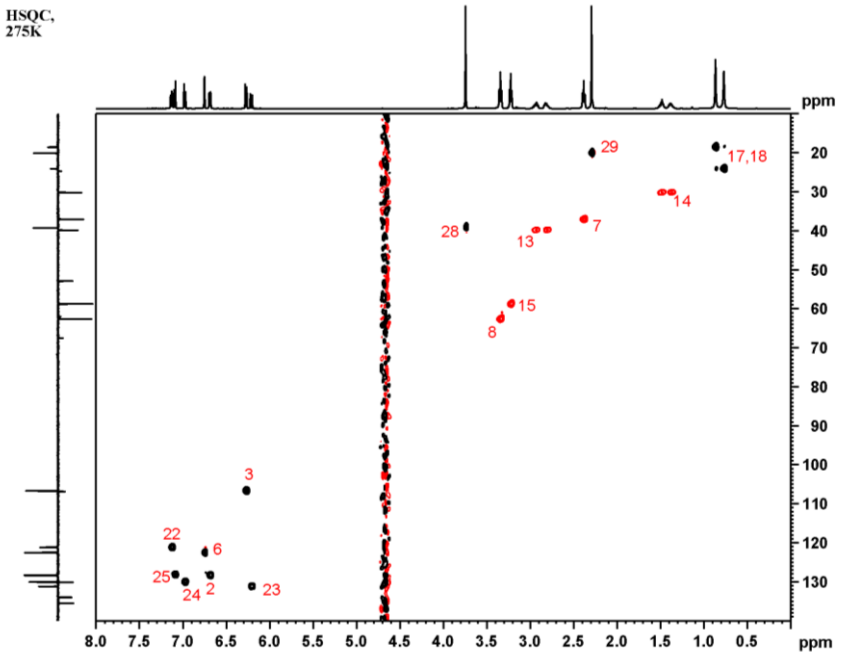
Supporting Figure 4. ^{1}H-1D spectra of compound **4** measured at 500 MHz, pH7 and 275K.



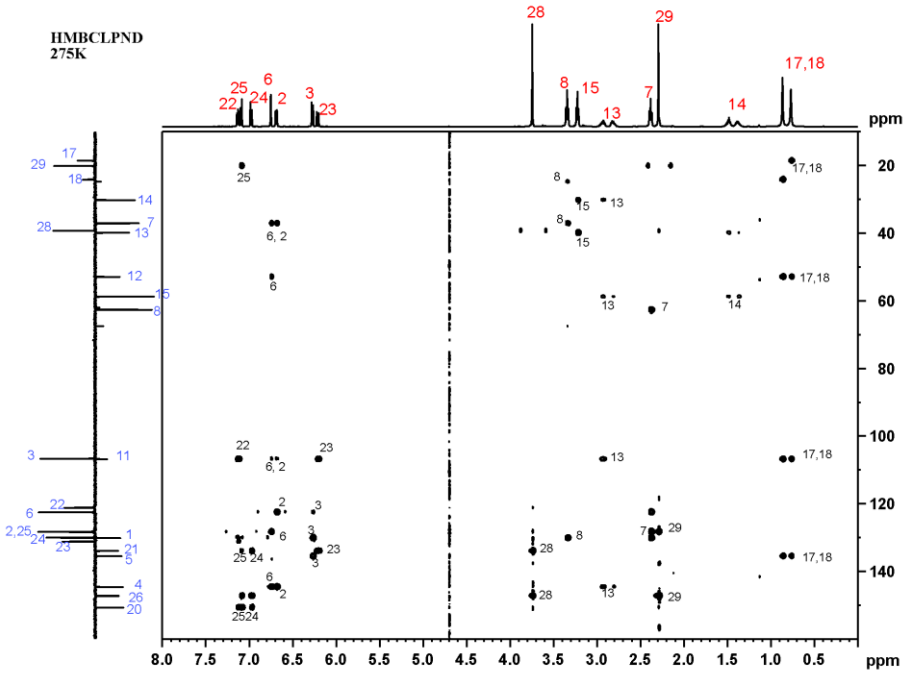
Supporting Figure 5. ^{13}C-1D DEPT-Q spectra of compound **4** measured at 500 MHz, pH7 and 275K. Positive signals show signals from CH and CH_3-groups; negative signals show C and CH_2 groups.



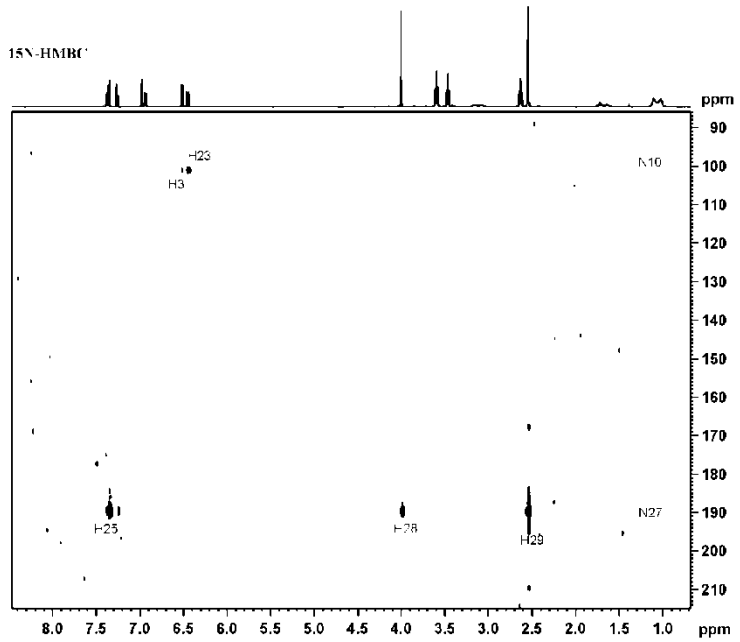
Supporting Figure 6. 2D ^{1}H, ^{1}H-ROESY of compound **4** measured at 500 MHz, pH7 and 275K. The ROESY mixing time was set to 300ms.



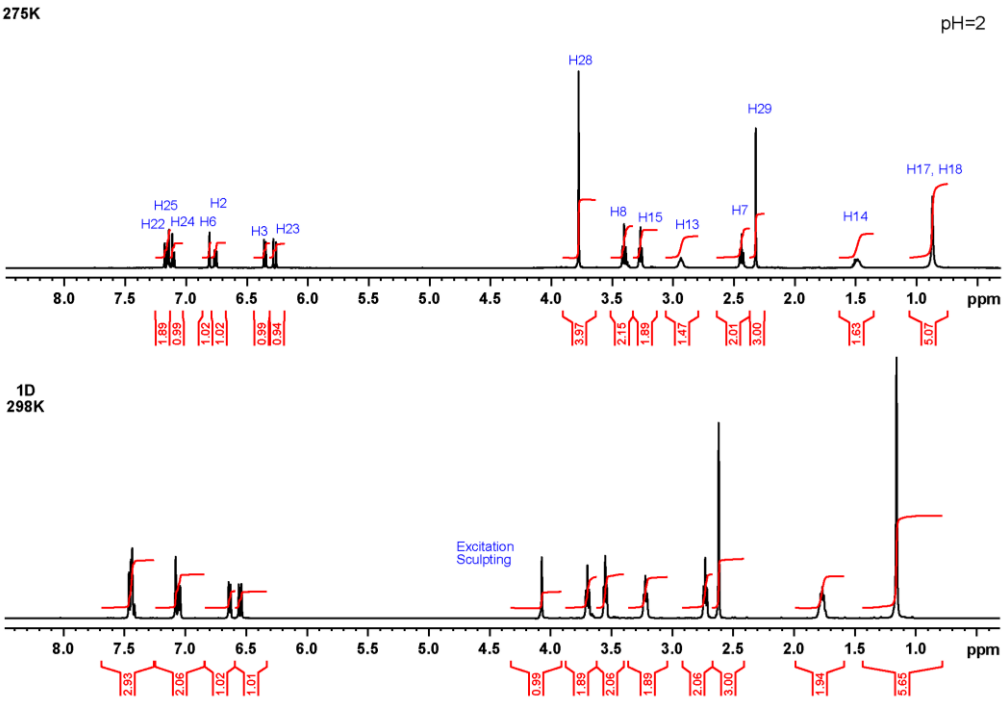
Supporting Figure 7. 2D ^{1}H, ^{13}C-editied HSQC of compound **4** (black: CH /CH_3; red: CH_2) measured at 500 MHz, pH7 and 275K.



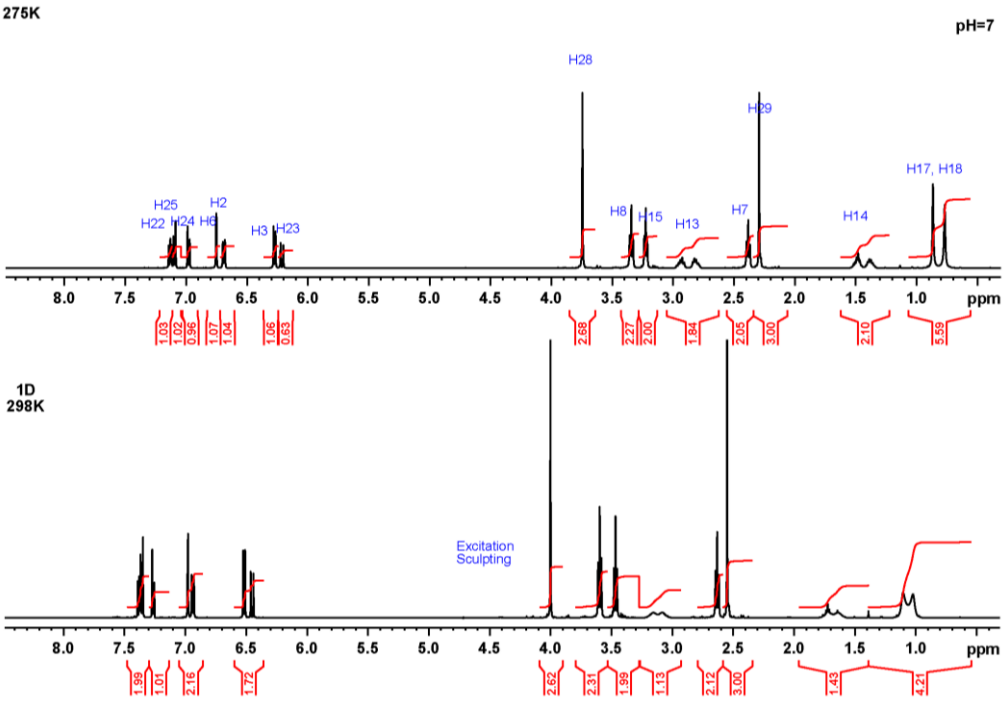
Supporting Figure 8. 2D ^{1}H, ^{13}C-HMBC of compound **4** measured at 500 MHz, pH7 and 275K.



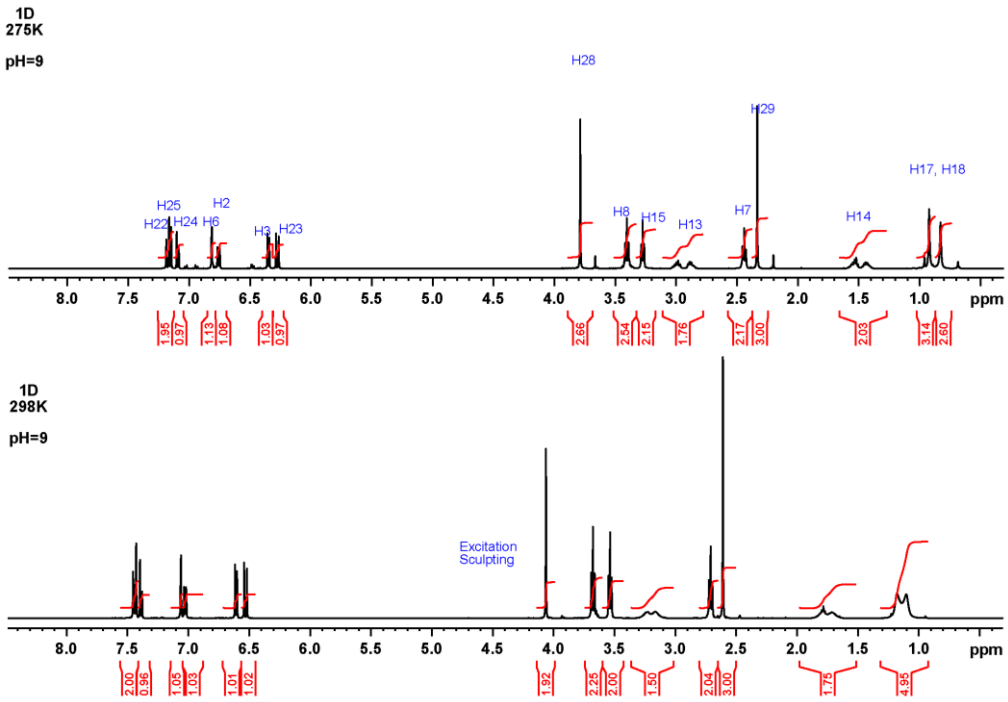
Supporting Figure 9. 2D ^{1}H, ^{15}N-HMBC of compound **4** measured at 500 MHz, pH7 and 275K.



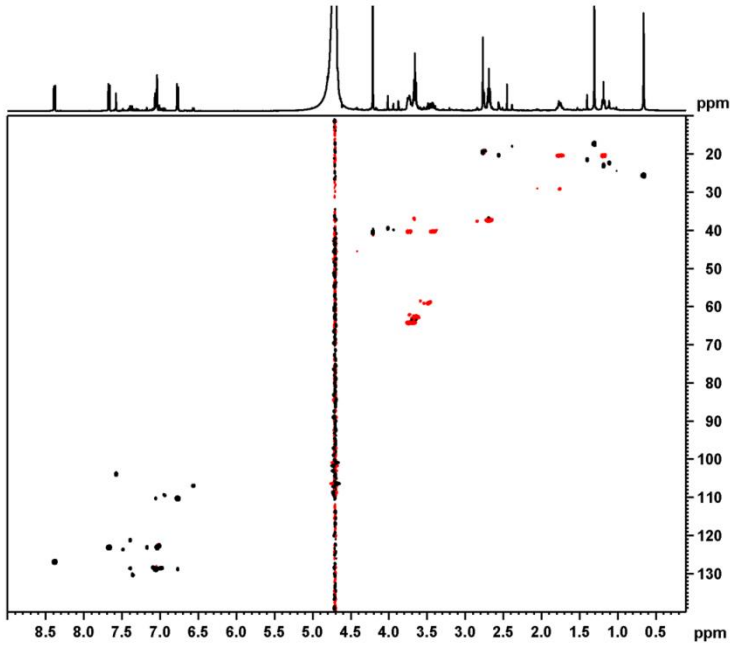
Supporting Figure 10. Comparison of ^{1}H-1D spectra of compound **4** measured at 275K and 298K, at pH2.



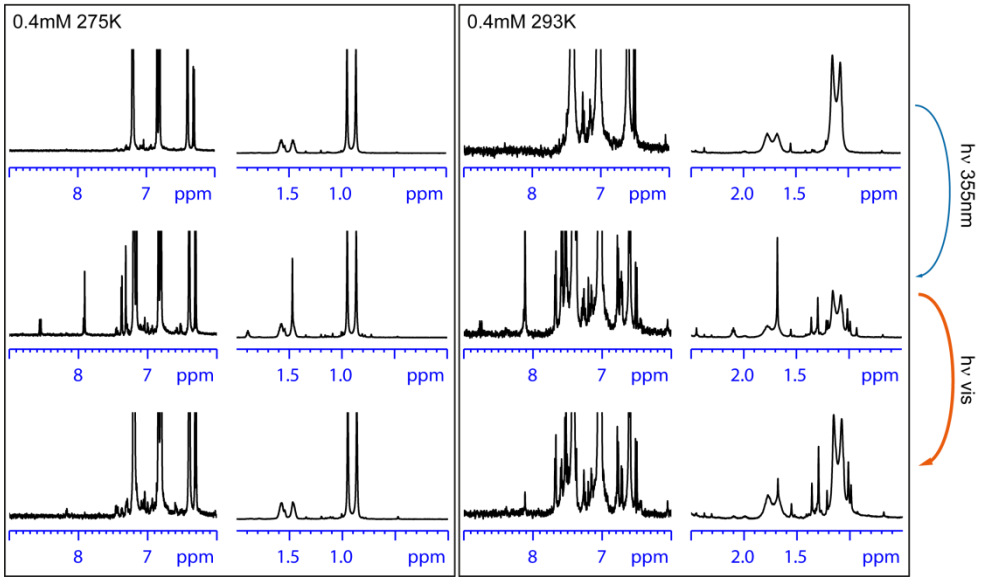
Supporting Figure 11. Comparison of ^{1}H-1D spectra of compound **4** measured at 275K and 298K, at pH7.



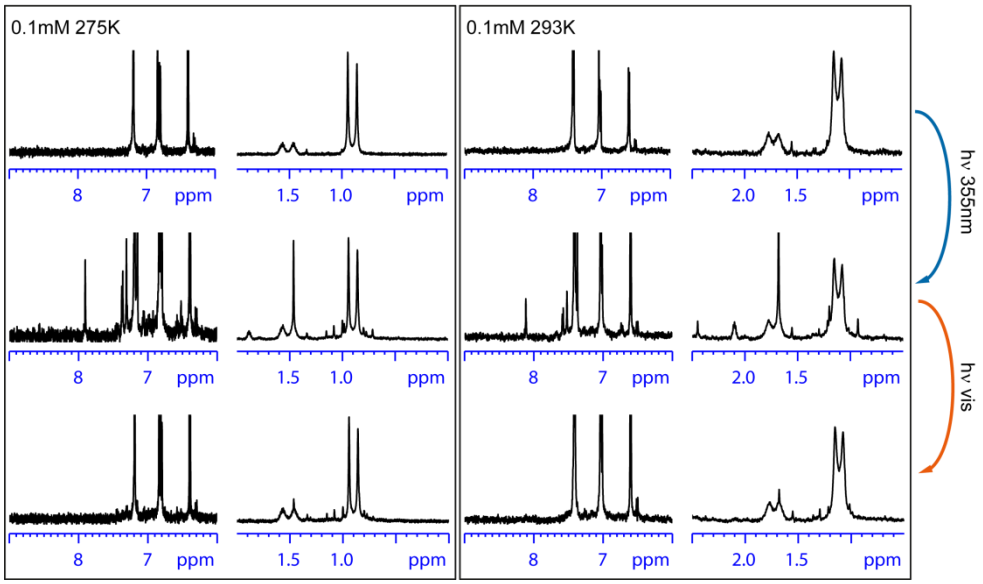
Supporting Figure 12. Comparison of ^{1}H-1D spectra of compound **4** measured at 275K and 298K, at pH9.



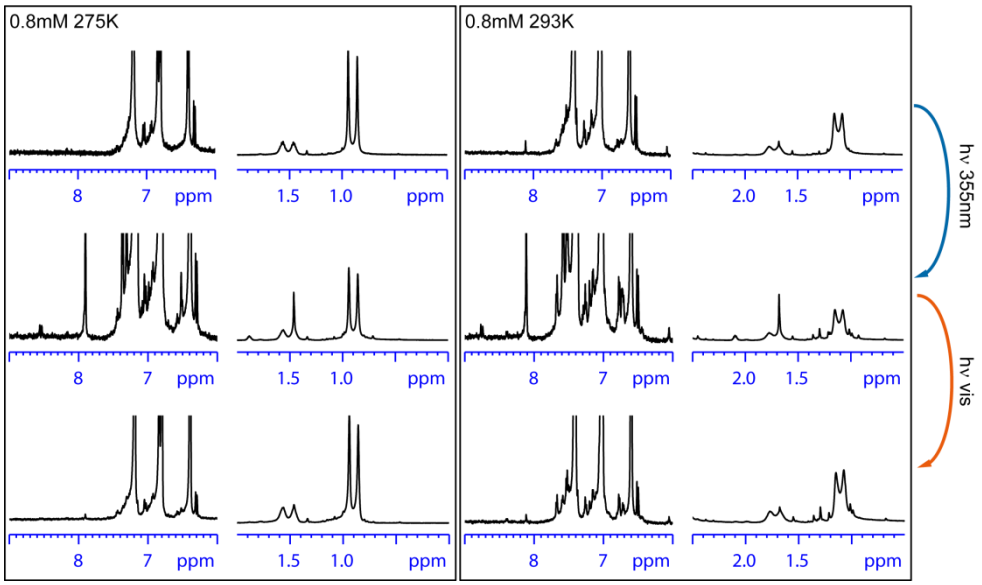
Supporting Figure 13. 2D ^{1}H, ^{13}C-edited HSQC of compound **4** (black: CH /CH_3; red: CH_2) measured at 500 MHz, pH7 and 298K after laser illumination.



Supporting Figure 14. Aromatic and methyl-region of 1D 1H NMR of compound **4** of spiropyran before UV, after UV and after VIS illumination at 0.4mM concentration and 275K and 293K.



Supporting Figure 15. Aromatic and methyl-region of 1D 1H NMR of compound **4** of spiropyran before UV, after UV and after VIS illumination at 0.1mM concentration and 275K and 293K.



Supporting Figure 16. Aromatic and methyl-region of 1D 1H NMR of spiropyran **4** before UV, after UV and after VIS illumination at 0.4mM concentration and 275K and 293K.

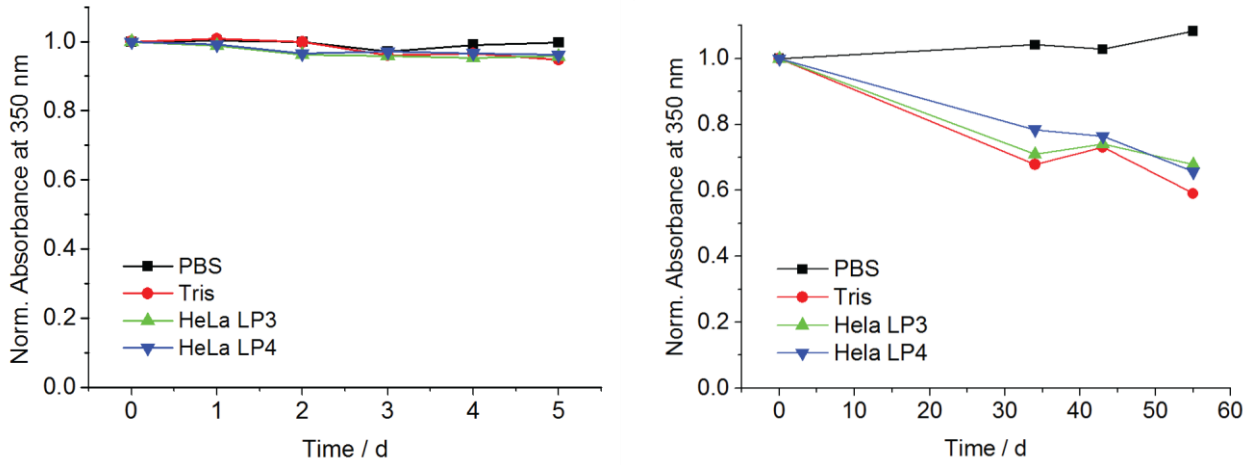
Aqueous Solubility

Supplementary Table 4. Comparison of solubilities in PBS buffer. c = maximal concentration

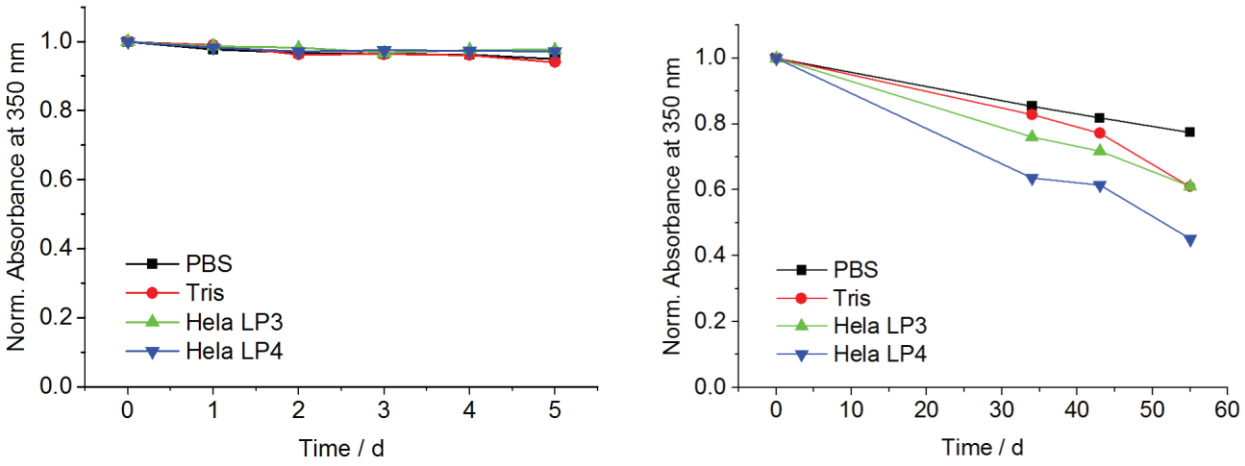
compound	c / (mM)
1a	< 0,05
1b	57
2	28
3	18
4	77

To compare the solubilities of different compounds in PBS buffer a defined amount of sample was dissolved in increasing amounts of PBS buffer (pH 7.4). After each addition of solvent the suspension was sonicated for 5 minutes and after centrifugation the sample was checked for residual solid material.

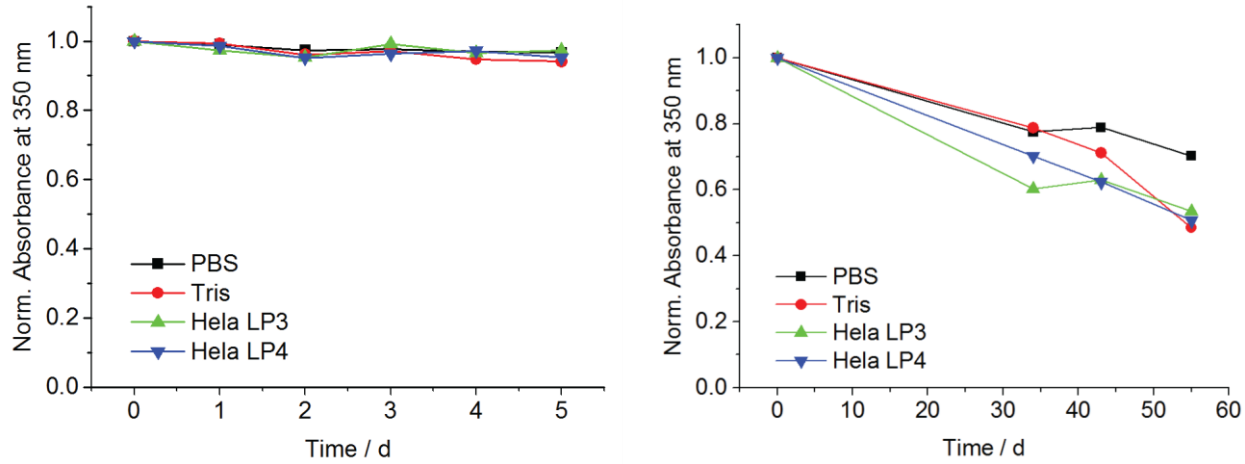
Hydrolysis Studies



Supporting Figure 17. Absorbance change of compound **2** at 350 nm in various solvent conditions over a period of 5 days (left) and over a period of 60 days (right).

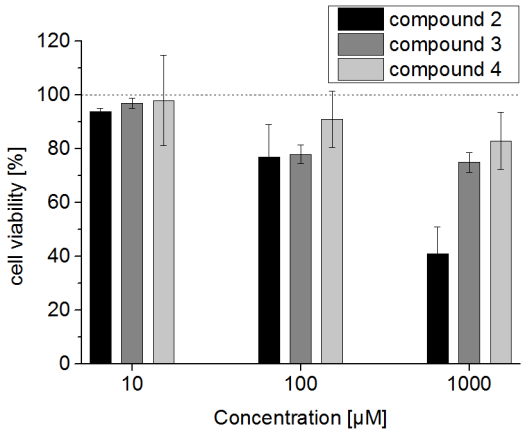


Supporting Figure 18. Absorbance change of compound **3** at 350 nm in various solvent conditions over a period of 5 days (left) and over a period of 60 days (right).



Supporting Figure 19. Absorbance change of compound **4** at 350 nm in various solvent conditions over a period of 5 days (left) and over a period of 60 days (right).

Cell viability studies



Supporting Figure 20. Influence of compounds **2**-**4** on HeLa cell viability. HeLa cells were seeded in DMEM at a density of 2,000 cells per well of a 96-well plate. After one day, cells were treated with the indicated compound for additional 24 hours. Cell proliferation was determined using WST-1 Cell Proliferation Reagent. Values are the mean of two independent experiments involving triplicates. The standard deviation is indicated.

Supporting References

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[S2] C. Petchprayoon, Y. Yan, S. Mao, G. Marriott, *Bioorg. Med. Chem.* **2011**, *19*, 1030–1040.

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