Electronic Supplementary Material (ESI) for Nanoscale.

Dissipative Self-Assembly of a Proline Catalyst for Temporal Regulation of the Aldol Reaction

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Synthetic Methods and Experimentals

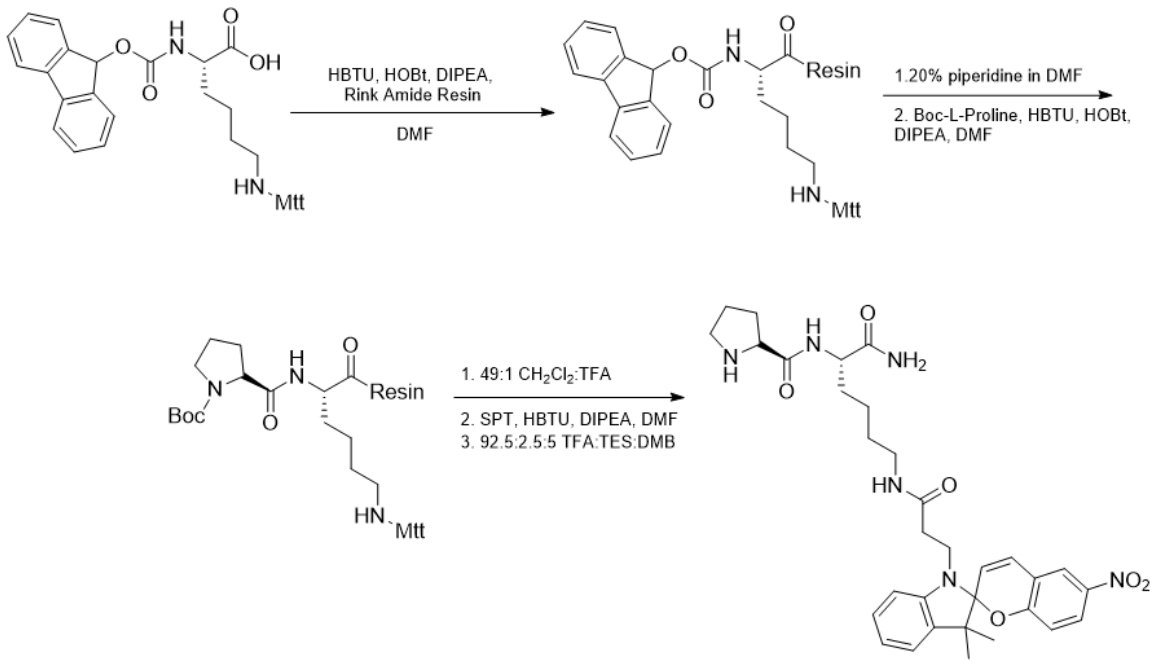
4-Azinobenzoic acid, 3-methyl-2-butanone, 2-hydroxy-4-nitrobenzaldehyde, piperadine, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), trifluoroacetic acid (TFA), triethylsilane (TES) and deuterated solvents were purchased from Oakwood Chemical and TCI America. Methyl iodide (MeI), N,N-diisopropylethylamine (DIPEA), solvents and acids were purchased from Fisher Scientific. The Fmoc-amino acids, and 1-hydroxybenzotriazole (HOBt), 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) were purchased from Chem-Impex Int'l Inc. 1,3-Dimethoxybenzene (DMB) and acetic anhydride was purchased from Sigma-Aldrich. Reverse-phase HPLC was performed on a ThermoScientific Ultimate 3000 HPLC using a Waters C8 XBridge 10 uM 9x10 mm prep. column w/ Guard Cartridge or a ThermoScientific C18 Acclaim120 5uM 4.6x100 mm analytical column. NMR spectra were obtained on Bruker AMX 400 spectrometers and masses on a Bruker microTOF ESI-MS. NMR spectra were obtained on Bruker AMX 400 spectrometers and masses on a Bruker microTOF ESI-MS. UV-Vis spectroscopy studies were conducted on a Shimadzu UV-2450 Spectrometer with a TCC-240A Temperature-Controlled Cell Holder using a 1 mm path length quartz cuvette. CD spectra were recorded on a Jasco CD spectrometer under a nitrogen atmosphere using a 1 mm path length quartz cuvette. Fluorescence spectra were recorded on a Shimadzu RF-5301 PC Spectrofluorometer using a 200 μL, 3 mm path length quartz cuvette. DLS measurements were obtained using a Malvern Zetasizer NanoZS system with low-volume quartz batch cuvette (ZEN2112).

Solid-Phase Peptide Synthesis (SPPS)

General Methods and Protocols. Peptide synthesis was performed on Rink amide solid-phase resin (~0.5 mmol/g loading) using fritted syringes with a Teflon stopcock and stoppered with a septum. A wrist-action shaker was used to agitate the syringes, and rinses were performed via vacuum filtration. Dry resin was allowed to swell in CH_2Cl_2 by shaking for 1 h before proceeding with the next step. A Kaiser test was performed after each coupling to ensure completion of each coupling step. Kaiser tests (ninhydrin tests) consist of three solutions. Solution A: Dissolved 16.5 mg KCN in 25 mL water. Diluted 1 mL of this solution into 48 mL distilled pyridine. Solution B: Dissolved 1 g ninhydrin in 20 mL *n*-butanol. Solution C: Dissolved 40 g phenol in 20 mL *n*-butanol. By placing a few resin beads in a test tube and adding ~1 drop of each solution and heating with a heat gun until just below boiling, the test shows a blue color when in the presence of free unreacted amines. Deprotection of the Fmoc protection groups was achieved using a 3 x 5 min treatment with piperidine (20%) in DMF. After each deprotection and coupling step, the resin was rinsed with DMF (3x), MeOH (2x), and CH_2Cl_2 (2x). Deprotection of the Mtt protecting groups was achieved by treatment with TFA (2%)/1% TES (1%) in CH_2Cl_2 for 5 min 3 times, followed by washing the resin with CH_2Cl_2 (3x). Simultaneous deprotection of the Boc group and cleavage of the peptide from the resin was achieved by treatment with 5% DMB (5%)/5% TES (5%)/TFA (90%) for 2 h. The solution was drained from the syringe and diluted with 100 mL of cold diethyl ether. Additional cold diethyl ether was added to induce precipitation of the crude peptide, which was then centrifuged (10 min, 6000 RPM) and the remaining supernatant removed by decanting. The crude peptide was dried by lyophilization then purified using prep-scale reverse-phase HPLC on C8 reverse-phase column using an CH_3CN/H_2O eluant, lyophilized, and stored at ambient temperature in darkness.

Peptide 1. The Rink resin (0.2 mmol, 1 eq) was swollen in CH_2Cl_2, and the Fmoc was deprotected using the standard protocol, described in the general methods. A solution of Fmoc-Lys(Mtt)-OH (0.38 g, 0.6 mmol, 3 eq) in 5:1 DMF:DCM was treated with HOBt (0.081 g, 0.6 mmol, 3 eq) and HBTU (0.23 g, 0.6 mmol, 3 eq) for 5 min., followed by DIPEA (0.14 mL, 0.8 mmol, 4 eq) for 1 min, which produced in a light yellow solution. The solution was then added to the resin, stoppered, and shaken for 14 h. Afterwards, the resin was washed with DMF (3 x 5 mL), MeOH (2 x 5 mL), and CH_2Cl_2 (2 x 5 mL), Fmoc-deprotected using a 3 x 5 min treatment with piperadine (20%) in DMF, and rinsed again with DMF (3 x 5 mL), MeOH (2 x 5 mL), and CH_2Cl_2 (2 x 5 mL). A solution of Pro(Boc)-OH (0.28 g, 0.6 mmol, 3 eq) in 5:1 DMF:CH_2Cl_2 was prepared in a separate vial, then HOBt (0.081 g, 0.6 mmol, 3 eq) and HBTU (0.23 g, 0.6 mmol, 3 eq) were added simultaneously. After 5 min., DIPEA (0.14 mL, 0.8 mmol, 4 eq) was added, resulting in a pale-yellow solution. The solution was then added to the resin, stoppered, and shaken for 14 h. Afterwards, the resin was washed with DMF (3 x 5 mL), MeOH (2 x 5 mL), and CH_2Cl_2 (2 x 5 mL), Fmoc-deprotected using a 3 x 5 min treatment with piperadine (20%) in DMF, and rinsed again with DMF (3 x 5 mL), MeOH (2 x 5 mL), and CH_2Cl_2 (2 x 5 mL). After rinsing the resin and deprotecting the Mtt group by treatment with TFA (2%)/1% TES (1%) in CH_2Cl_2 for 5 min 3 times, followed by washing the resin with CH_2Cl_2 (3 x 5 mL), a solution of 3-(3',3'-dimethyl-6-nitrospiro[chromene-2,2'-indolin]-1'-yl)propanoic acid (**SPT-CO_2H)**[1] (0.22 g, 0.6 mmol, 3 eq) in 5:1 DMF:CH_2Cl_2 was prepared in a separate vial and treated with HOBt (0.081 g, 0.6 mmol, 3 eq) and HBTU (0.23 g, 0.6 mmol, 3 eq) for 5 min, followed by DIPEA (0.14 mL, 0.8 mmol, 4 eq), resulting in purple-red solution. After 1 min., the solution was then added to the resin, stoppered, and shaken for 14 h. After rinsing with DMF (3 x 5 mL), MeOH (2 x 5 mL), and CH_2Cl_2 (2 x 5 mL), the Boc-protecting groups were removed and the peptide was cleaved simultaneously from

the resin by treatment with 5 mL of 5% DMB (5%)/5% TES (5%)/TFA (90%) for 2 h. The crude peptide was precipitated in 40 mL cold diethyl ether, centrifuged and purified to yield **1**. Purification performed with preparatory scale reverse-phase HPLC on a C8 reverse-phase column with CH_3CN/H_2O with 0.1% TFA as eluant using the following gradient: 8 mL/min; 1 min. at 20% CH_3CN; 1-12 min up to 42% CH_3CN; 12-32 min up to 50% CH_3CN; 32-33 min up to 100% CH_3CN; 33-34 min at 100%; 34-35 min down to 25% CH_3CN. Two peaks were isolated (18 min, **1**-MCH^+ **(**trace**)**; 28 min, **1**-SP). **1**-SP ^1H NMR (600 MHz, DMSO-d_6): δ 9.15 (bm, 1H, proline ammonium NH), 8.53 (d, *J*= 11.9 hz, NH, exchangeable), 8.49 (bm, 1H, proline ammonium NH), 8.22 (d, *J* = 2.8 Hz, 1H), 8.00 (dd, *J* = 9.0, 2.8 Hz, 1H), 7.88 (t, *J* = 5.4 Hz, 1H, NH, exchangeable), 7.44 (s, 1H, NH_2, exchangeable), 7.20 (d, *J* = 10.3 Hz, 1H), 7.13 (m, 2H), 7.05 (s, 1H, NH_2, exchangeable), 6.86 (d, *J* = 9.1 Hz, 1H), 6.80 (td, *J* = 7.5, 0.8 Hz, 1H), 6.66 (d, *J* = 7.7 Hz, 1H), 5.98 (d, *J* = 10.4 Hz, 1H), 4.22 – 4.16 (m, 2H), 3.46 (m, 1H), 3.34 (m, 1H), 3.27 – 3.14 (m, 2H), 2.97 – 2.90 (m, 2H), 2.39 (m, 1H), 2.33 – 2.24 (m, 2H), 1.92 – 1.81 (m, 3H), 1.62 (m, 1H), 1.51 (m, 1H), 1.38 – 1.20 (m, 4H), 1.19 (s, 3H), 1.08 (s, 3H).^{13}C NMR (600 MHz, DMSO-d_6): δ = δ 173.32, 170.81, 168.31, 159.70, 146.79, 140.96, 136.08, 128.45, 128.03, 123.19, 122.40, 122.16, 119.62, 119.35, 115.95, 107.10, 107.07, 59.27, 53.19, 53.10, 52.92, 46.10, 38.76, 35.28, 32.03, 29.90, 29.17, 25.98, 23.84, 23.21, 19.87 ppm. ESI-MS Calculated for $C_{32}H_{41}N_6O_6$ $[M+H]^+$ = 605.3083, found = 605.3094.



Scheme S1. Solid-phase synthesis of dipeptide **1.**

General Methods

Microscopy

Transmission Electron Microscopy (TEM) images were obtained using FEI Tecnai G2 Biotwin TEM- negative staining. Samples were prepared by placing a drop of sample on a clean parafilm surface, floating a copper 200 mesh TEM grid (Ted Pella, Inc.) on top for 2-3 min, then removing the excess liquid with a clean Kimwipe. Staining with 2% aqueous uranyl acetate (filtered through a 0.2 ɥm syringe filter) was performed by placing a drop of stain solution on a clean parafilm surface then floating the sampled copped grid on top for 40-50 seconds then removing the excess liquid with a clean Kimwipe.

Atomic Force Microscopy (AFM) images were collected on a NanoScope IIIa device at ambient temperature. 0.3 mM diluted sample solutions (diluted from 3 mM stock assembled solns.) were dropped on freshly cleaved mica and allowed to dry for 10 min, then washed with water- the excess dabbed off with the side of a Kimwipe. Samples were allowed to fully dry. The AFM tip was a Model: SCANASYST-AIR from Bruker with a silicon tip on nitride lever; cantilever T- 600nm. The scanning speed was at a line frequency of 1.0 Hz, and the original images were sampled at a resolution of 512 × 512 pixels. Nanoscope software was used to analyze images.

Dynamic Self-Assembly

For room temperature preparations in the light or dark, a polypropylene box internally covered in mirrors was designed and built to house eight LED lights along the sides (3W, 270 lumens) and four shorter LED lights on the back wall (2W, 150 lumens) to irradiate the vials containing solutions of **1**-SP/MCH^+ (Fig. S1). For experiments above room temperature, the same propylene lightbox was used with a small hotplate and oil bath filled with colorless and very clear silicone oil. Lastly, a front cover mirror was closed to prevent light from entering during dark experiments. The LED lights were connected to an external switch for ease of turning on or off.

General Procedure for Aldol Reaction

Solid peptide monomer **1** (1 eq.) was added to a solution of an acid in H_2O (0.1 M). After stirring at 60°C for 20 min to ensure no assemblies from the solid state remained, the solution was added to a mixture of 4-nitrobenzaldehyde (5 eq.) and cyclohexanone (50 eq.) were added. The resulting mixture was stirred at 60°C for the indicated time. This mixture was then diluted with an equal

volume of water, and extracted with ethyl acetate (3 x 3 mL). The combined organic layers were then concentrated in vacuo and the resultant residue was purified by HPLC to give pure aldol diastereomeric products. The diastereomeric ratios were calculated based on integrations of the diagnostic signals of the *syn* and *anti* diastereomers in the ^{1}H NMR spectra of the crude reaction mixture. The enantioselectivities were calculated from HPLC profiles of the reaction mixtures, see below; the % e.e.s were reported the major *anti* diastereomer.

Optimized procedure for aldol reaction of cyclohexanone with 4-nitrobenzaldehyde

Solid peptide monomer **1** (1.26 mg, 0.003 mmol, 5 mol%) was added to a solution of 50 mM acid in H_2O (0.42 mL). After stirring at 60° C for 20 min to convert peptide **1** into **1**-MCH^+ and dissociate any aggregates of **1**, the solution was added to a mixture of 4-nitrobenzaldehyde (0.021 mL, 0.3 mmol, 5 eq.) and cyclohexanone (6.3 mg, 0.06 mmol, 50 eq.). The resulting mixture was stirred at 60°C under continuous irradiation with visible light from LEDs (72W, 3600 lumens). After 16 h, the mixture was diluted with water (0.42 mL) and extracted with ethyl acetate (3 x 3 mL). The combined organic layers were then concentrated in vacuo and the resultant residue was purified by HPLC (preparatory reverse-phase HPLC with a C8 column using CH_3CN:H_2O with 0.1% TFA as eluant using the following gradient: 10 mL/min; 1 min at 20% CH_3CN; 1-12 min up to 42% CH_3CN; 12-32 min up to 50% CH_3CN; 32-34 min up to 100% CH_3CN; 34-35 min down to 25% CH_3CN) to give pure aldol products: 2-[hydroxy-(4-nitrophenyl)-methyl]-cyclohexanone (89% conversion), as a white solid. The diastereoselectivity was determined by integration of the ^{1}H NMR peaks at 4.83 ppm (*anti*) and 5.42 ppm (*syn*) to be 2.69:1 (*anti:syn*). The enantioselectivity was determined by chiral HPLC Daicel Chiralpak AD-RH, 20% CH_3CN:H_2O with 0.1% TFA using the folowing gradient 1 mL/min; 40 min at 40% CH_3CN; 40 to 60 min up

to 50% CH_3CN; 60 to 90 min up to 60% CH_3CN, UV 254 nm. 73% ee for *anti* isomer (major, *R* isomer), ^tR 31.2 min, (minor, *S*-isomer), ^tR 53.2 min. *Syn* isomers, ^tR 24.5 min, ^tR 61.7 min (Figure S2).

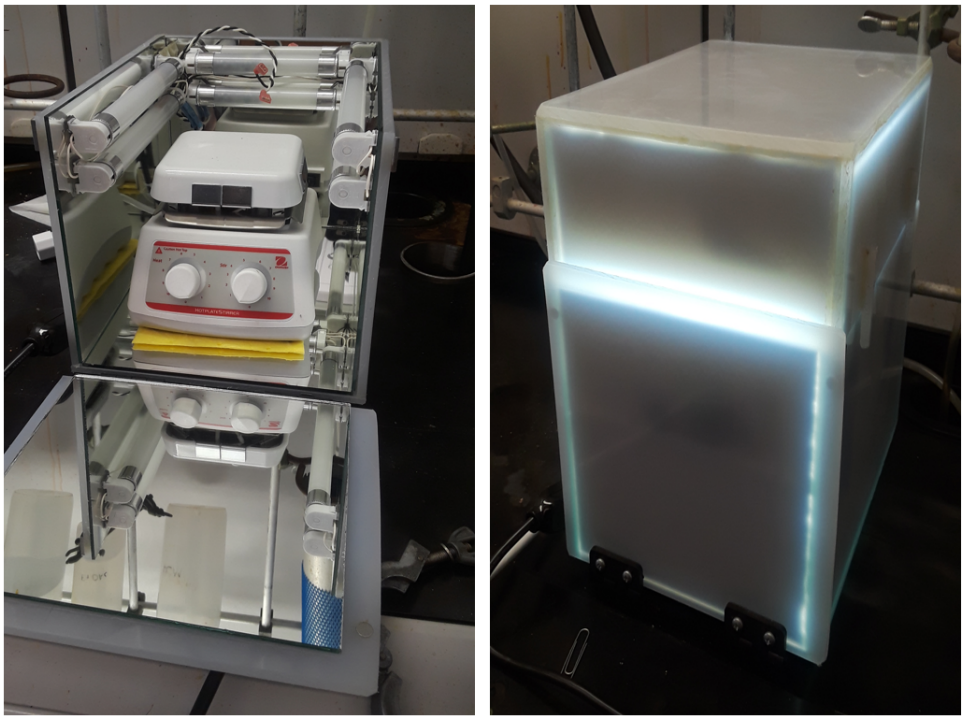


Figure S1. Light box that was designed and used for catalysis experiments. Housing made of chemically resistant plastic, with removable lid and remotely switchable LED bulbs lining three sides of the box. In total there were twelve LED bulbs, with eight 3W 270 lm bulbs, and four 2W 150 lm bulbs.

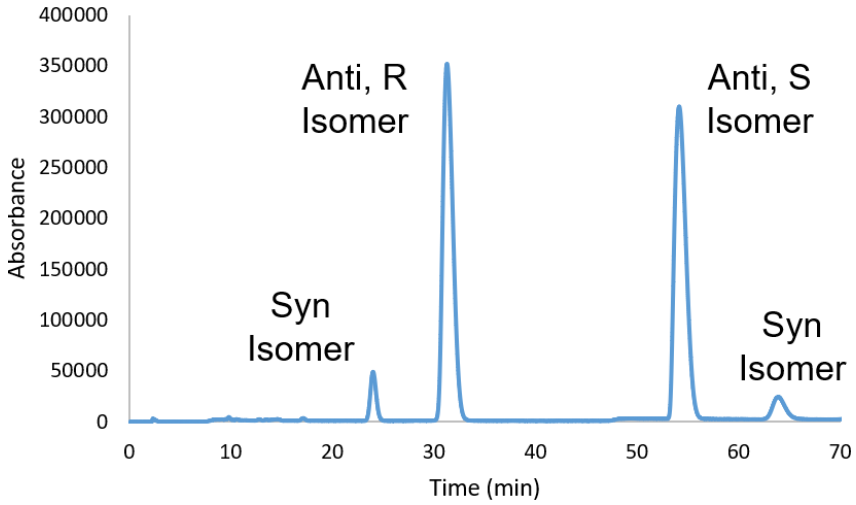


Figure S2. Representative resolution of racemic mixture of aldol products by HPLC. Chiral HPLC performed on a Daicel Chiralpak AD-RH, 20% CH_3CN:H_2O with 0.1% TFA 1 mL/min; 40 min at 40% CH_3CN; 40 to 60 min up to 50% CH_3CN; 60 to 90 min up to 60% CH_3CN, UV 254 nm. *Anti* isomers (R isomer) ^tR 31.2 min, (S-isomer), ^tR 53.2 min. *Syn* isomers, tR 24.5 min, tR 61.7 min. Racemic aldol product was obtained by combining 4-nitrobenzaldehyde (2 g, 1 eq) and cyclohexanone (13.8 mL, 10 eq) in H_2O (132 mL, 1M) with NaOH (5.2 g) as catalyst at room temperature. After 2 h, the reaction mixture was extracted with ethyl acetate (3 x 40 mL) and the combined organic layers were washed with water (10 mL), brine (10 mL), dried over Na_2SO_4 and concentrated in vacuo. Residue was purified by column chromatography on silica gel (20-40% EtOAc/hexanes) to give the aldol products: 2-[hydroxy-(4-nitrophenyl)-methyl]-cyclohexanone (3.8 g, 15.3 mmol, 71%), as an orange solid. *Syn* and *anti*-diastereomers were analyzed via HPLC to determine retention times of all 4 stereoisomers.

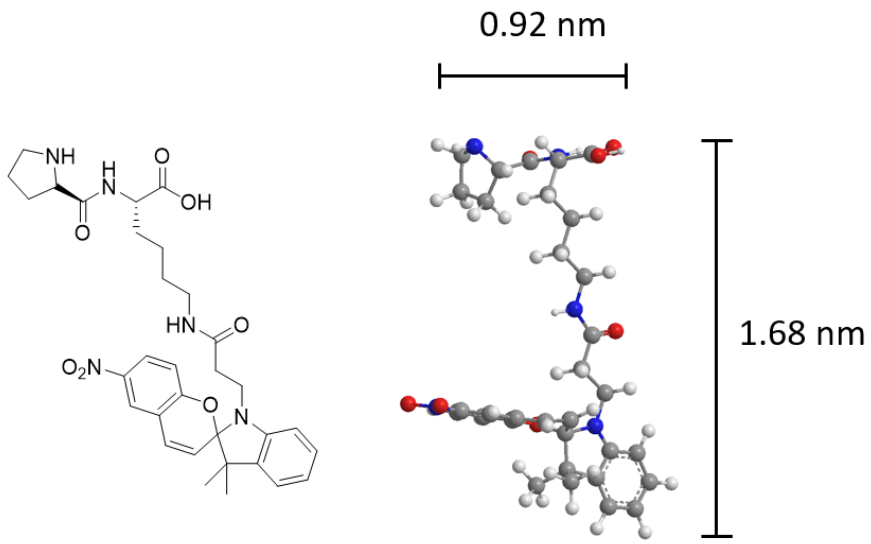


Figure S3. Extended dimensions of **1**-SP, modeled using Chem 3D.

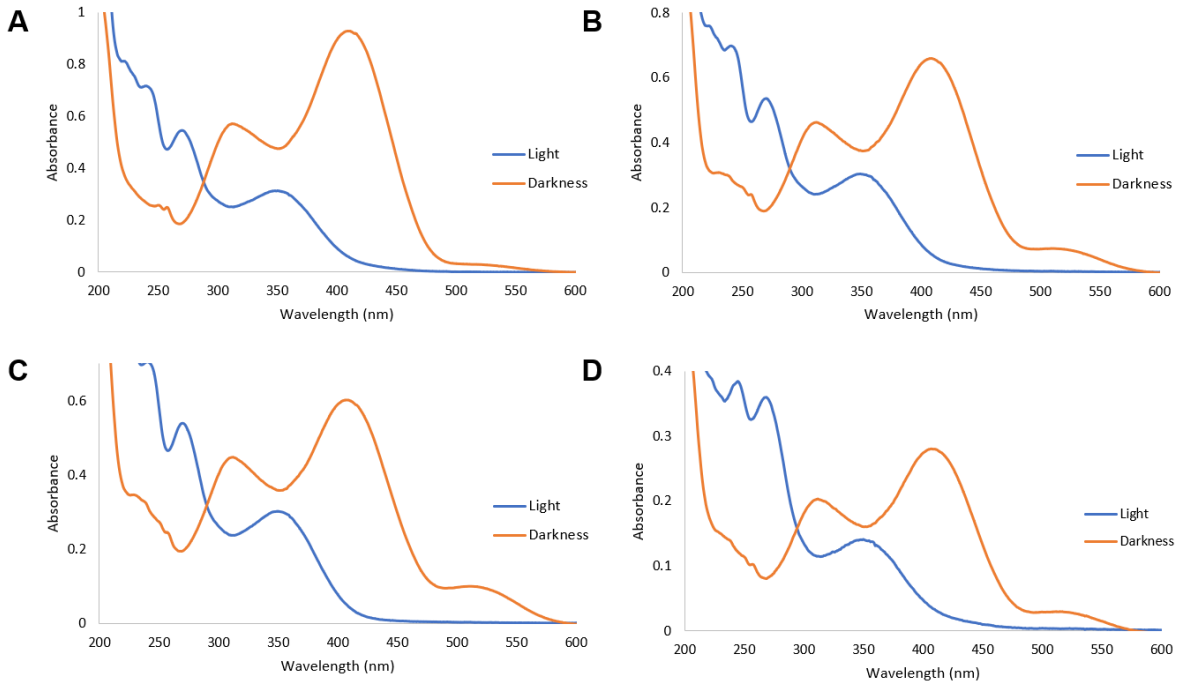


Figure S4. UV-Vis spectra of **1** in water with 50 mM of added acid: A) HCO_2H, B) CH_3CO_2H, C) $C_2H_5CO_2H$, D) $C_4H_7CO_2H$. Spectra were taken of **1** in H_2O (0.5 mM) with specified acid additive (50 mM) after incubate at 60°C for 40 minutes under continuous irradiation with visible light or under dark conditions.

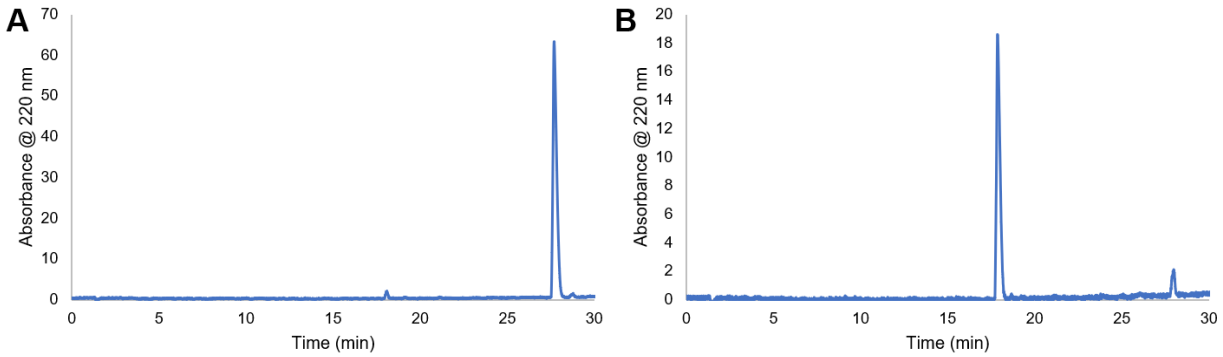


Figure S5. HPLC traces of a 0.5 mM solution of **1** in water with 50 mM HCO_2H. A) irradiated with visible light for 10 minutes at room temperature and B) heated at 60 °C for 45 minutes. The peak at 18 minutes corresponds to the **1**-MCH^+ form, and the peak at 28 minutes corresponds to **1**-SP. Collected using analytical-scale reverse-phase HPLC with a C18 MeCN:Water with 0.1% TFA (1mL/min; 1 min at 5%; 1-30 min up to 100%; 30-32 min down to 20%).

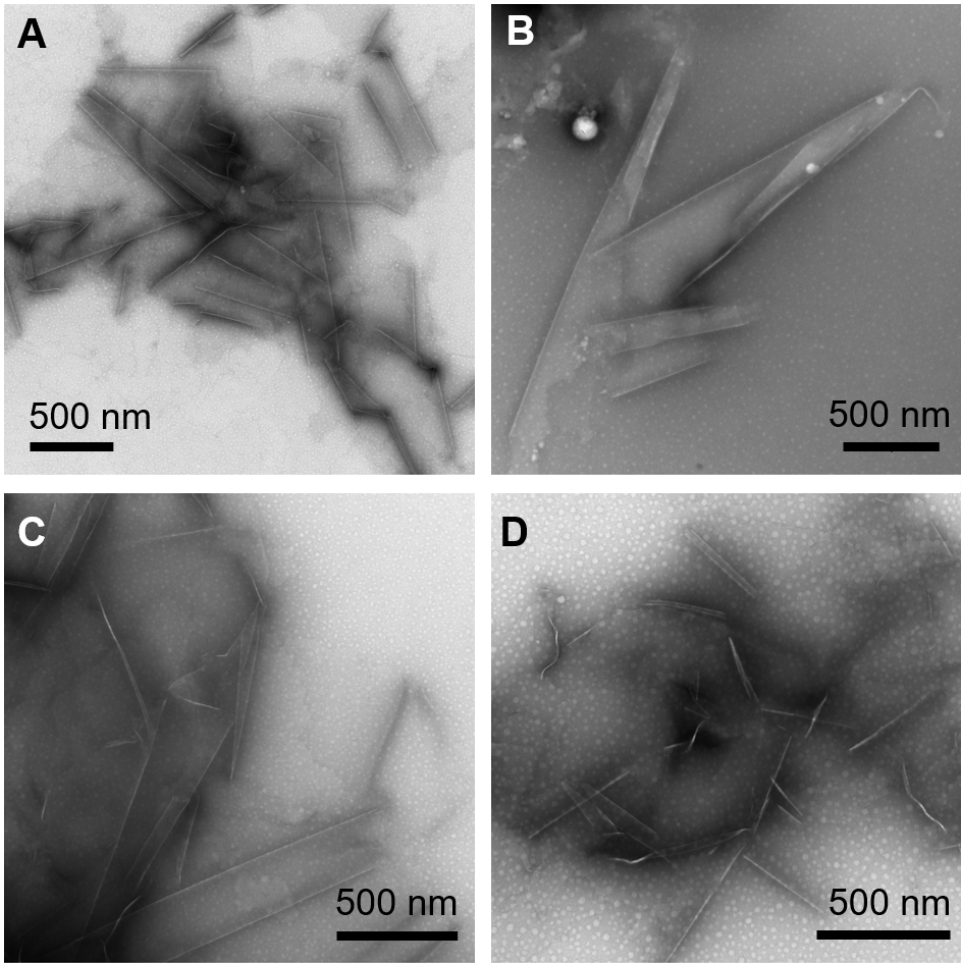


Figure S6. TEM images of **1** in water (5 mM) with 50 mM of A) HCO_2H, B) CH_3CO_2H, C) $C_2H_5CO_2H$, D) $C_4H_7CO_2H$. Samples incubated at 60ºC for 2 h at 5 mM while continuously irradiated with visible light. Samples stained with 2% aqueous uranyl acetate.

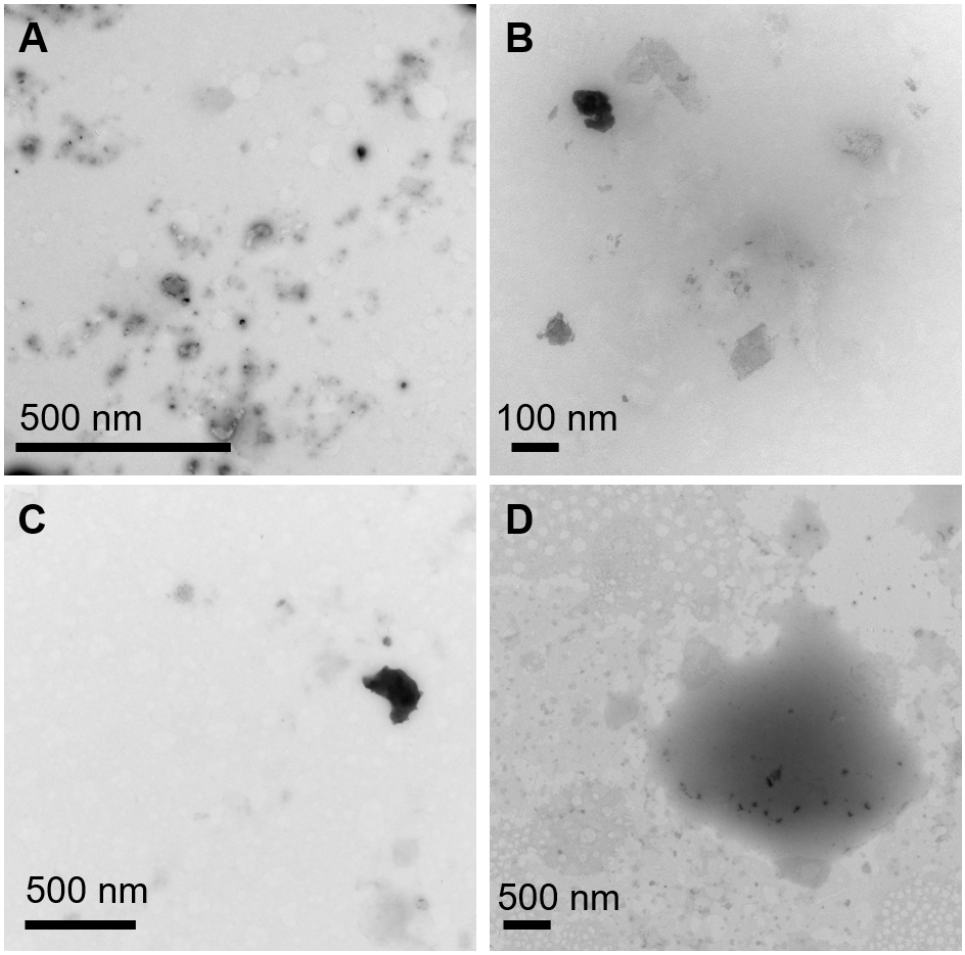


Figure S7. TEM images of **1** in water with 50 mM of A) HCO_2H, B) CH_3CO_2H, C) $C_2H_5CO_2H$, D) $C_4H_7CO_2H$. Samples incubated at 60ºC for 2 h in darkness. Stained with 2% aqueous uranyl acetate.

Trial	Photochemical conditions	Acid (50 mM)	Scattering (10^4 counts s^{-1} M^{-1})
1	Light	HCO_2H	451 ± 8
2	Darkness	HCO_2H	85 ± 26
3	Light	CH_3CO_2H	441 ± 45
4	Darkness	CH_3CO_2H	100 ± 5
5	Light	$C_2H_5CO_2H$	466 ± 28
6	Darkness	$C_2H_5CO_2H$	58 ± 14
7	Light	$C_4H_7CO_2H$	477 ± 28
8	Darkness	$C_4H_7CO_2H$	76 ± 22

Table S1. Dynamic light scattering studies for a series of experiments with **1** using varying acids. Samples were prepared by dissolving **1** in H_2O (5 mM) with indicated acid additive (50 mM) and heating at 60°C for 2 h in light or darkness. Samples were prepared in triplicate and the molar scattering intensities were recorded using a Malvern Zetasizer NanoZS system with low-volume quartz batch cuvette.

	Photochemical conditions	Acid	Temp	Conversion
1	Light	HCO_2H (50 mM)	60 °C	0%
2	Darkness	HCO_2H (50 mM)	60 °C	0%
3	Light	HCO_2H (10 mM)	40 °C	0%
4	Darkness	HCO_2H (10 mM)	40 °C	0%
5	Light	TFA (10 mM)	40 °C	0%
6	Darkness	TFA (10 mM)	40 °C	0%
7	Light	CH_3CO_2H (10 mM)	40 °C	0%
8	Darkness	CH_3CO_2H (10 mM)	40 °C	0%

Table S2. Controls performing the reaction of 4-nitrobenzaldehyde and cyclohexanone in absence of **1** as a function of acid additive and temperature. Reactions were conducted for 16 h using a 1:10 aldehyde/ketone ratio using the indicated acid in water under light-on and light-off conditions. No measurable conversion of starting material via NMR was observed for any conditions lacking **1**.

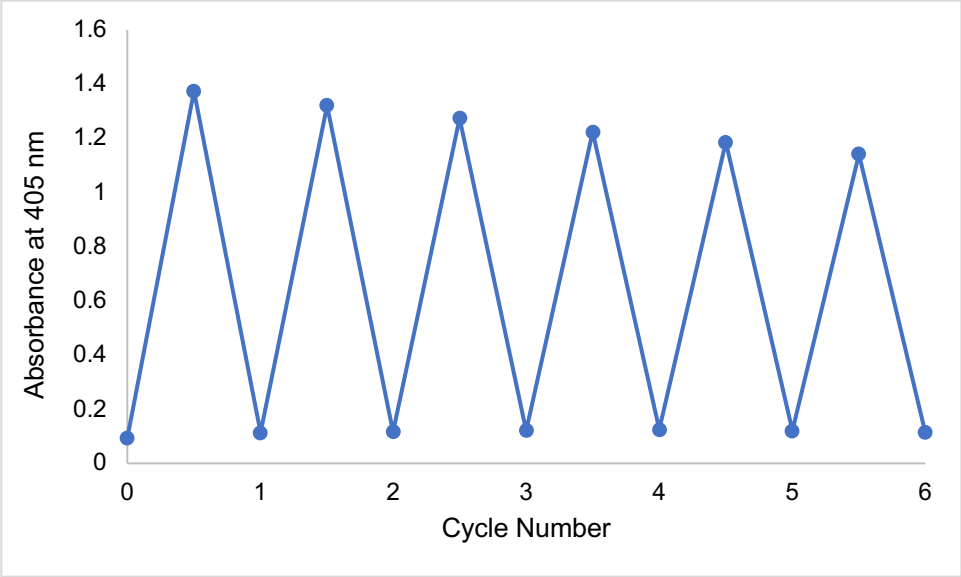


Figure S8. A solution of **1** (1 mM) in water (50 mM HCO_2H) subjected to several 5-minute cycles of irradiation with visible light at 60 °C to form **1**-SP followed by 40 minutes at 60°C with the light off (dark) to form **1**-MCH. After 6 cycles of **1**-SP/**1**-MCH interconversion, a 17% decrease in the maximum absorbance was observed at 405 nm.

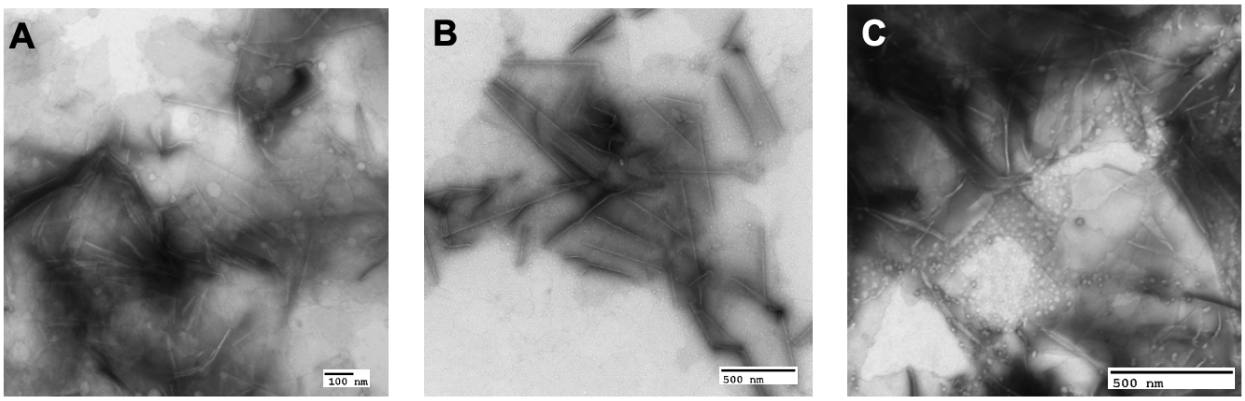


Figure S9. TEM images of **1** (1 mM) and heating in water (50 mM HCO_2H) to 60°C under irradiation with visible light for (A) 1 h, (B) 2h and (C) 16 h.

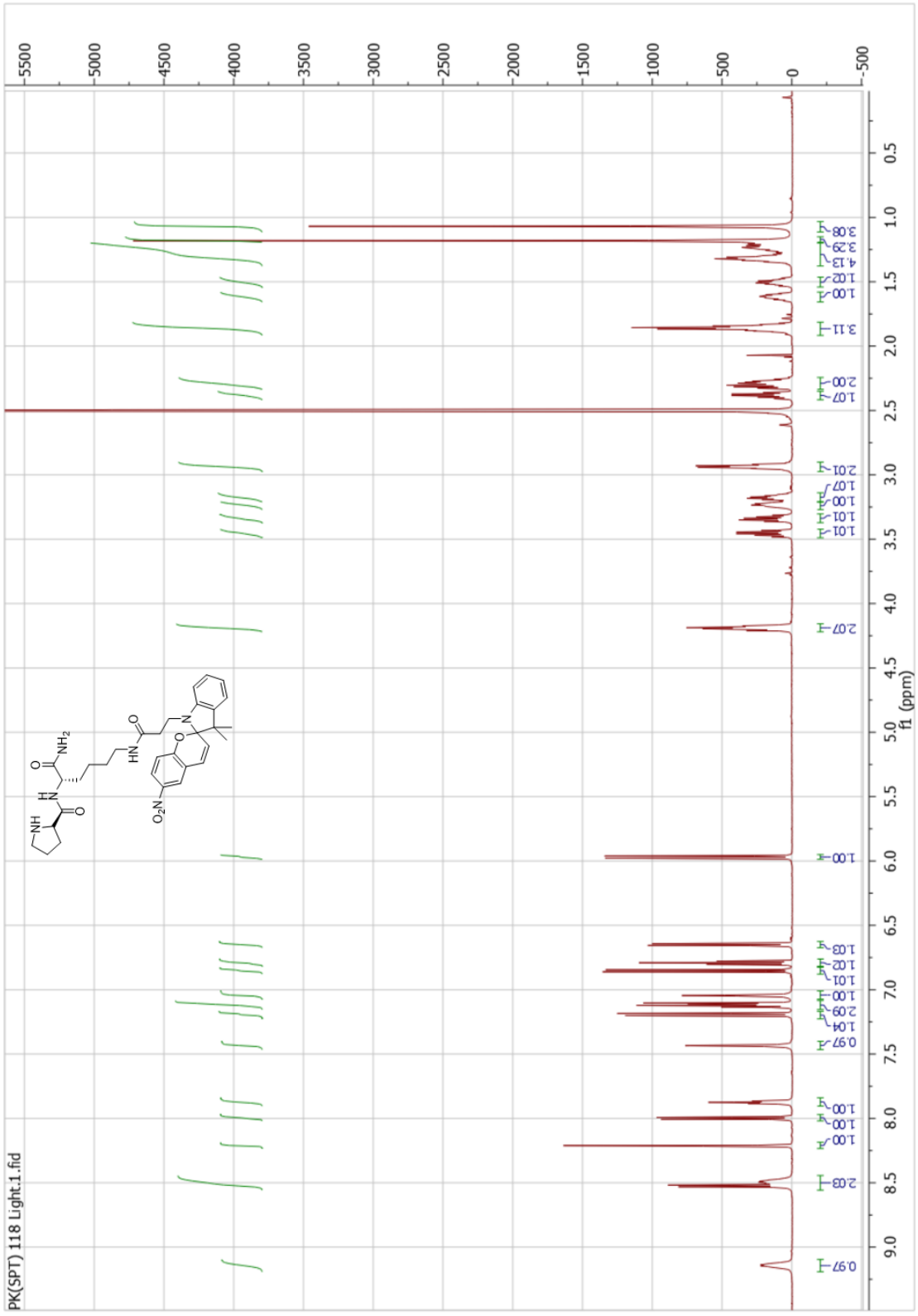


Figure S10. ^{1}H NMR spectrum of **1**-SP in DMSO-d_6.

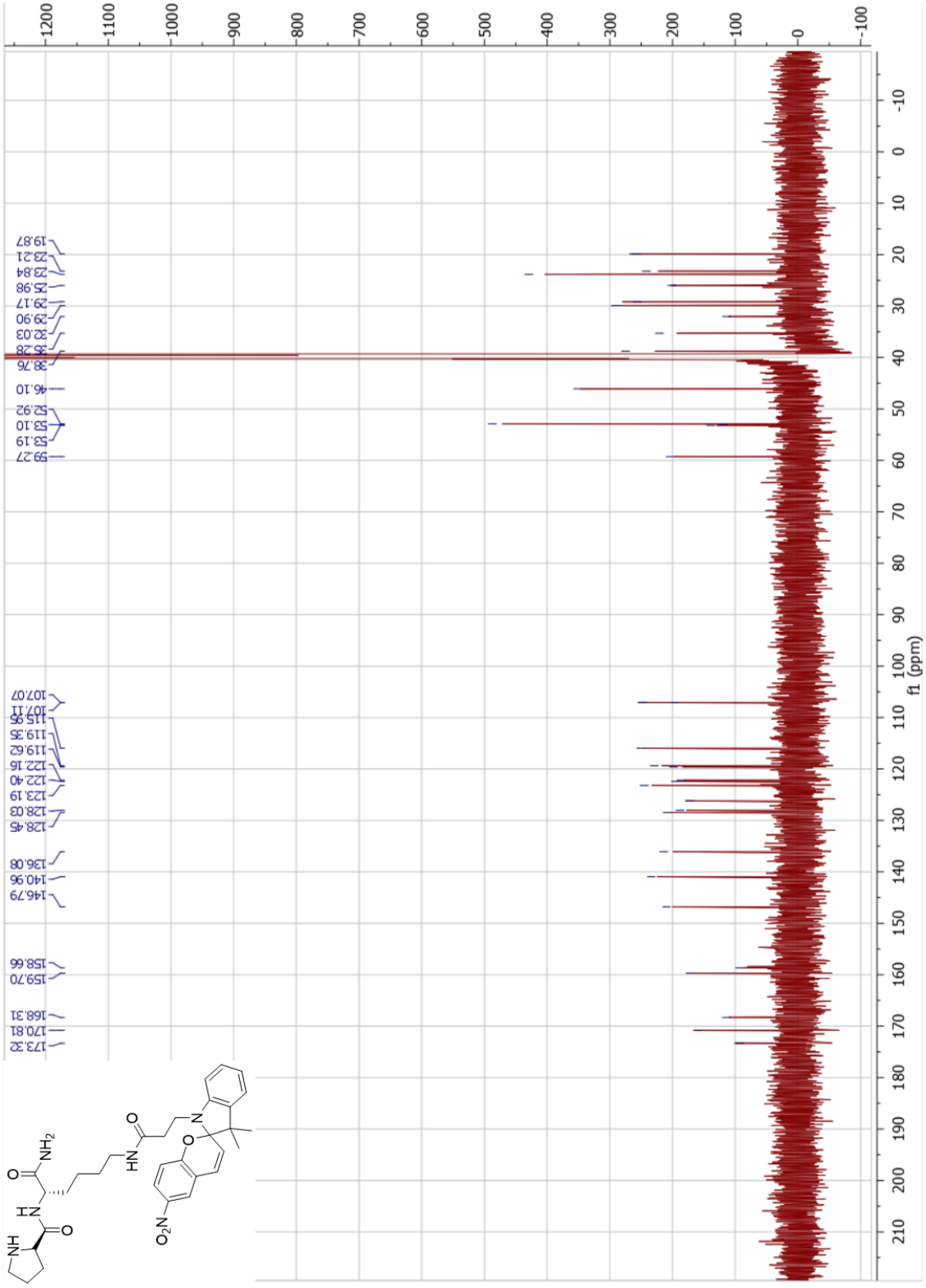


Figure S11. ^{13}C NMR spectrum of **1**-SP in DMSO-d_6.

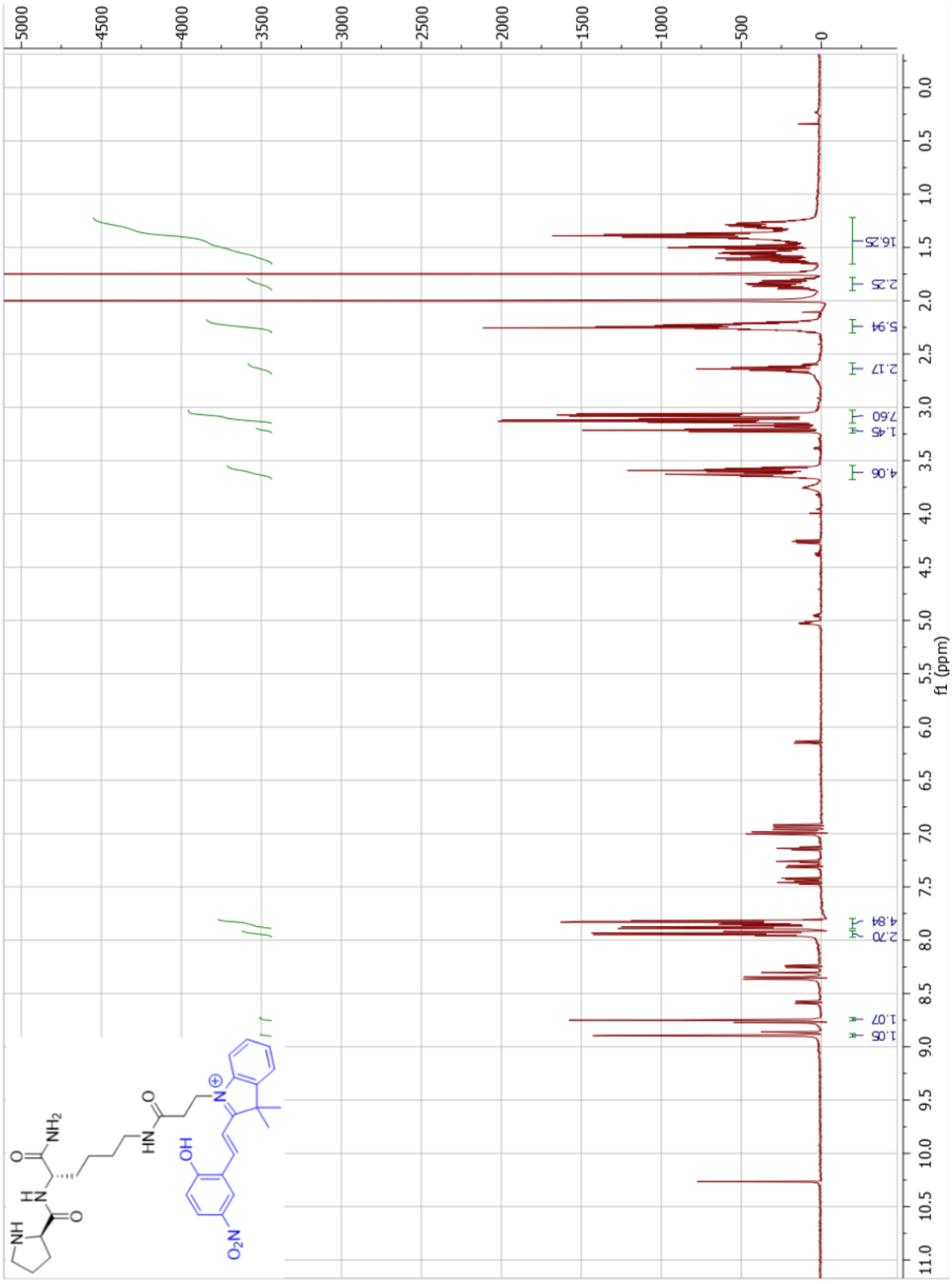


Figure S12. ^{1}H NMR spectrum of **1**-MCH^{+} in DMSO-d$_6$, obtained by heating **1**-SP in D$_2$O at 60°C. Minor amounts of **1**-SP are present in the spectrum.

ESI-MS Analysis

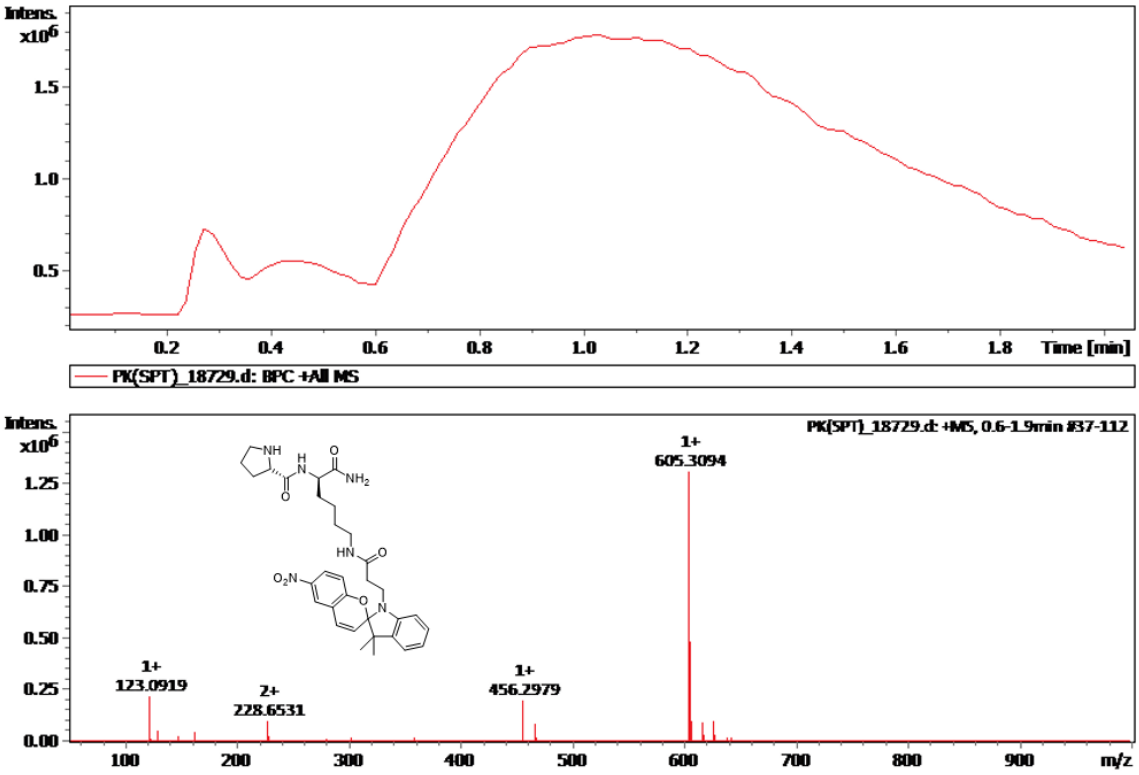


Figure S13. High resolution ESI-mass spectrum of **1**-SP.

References

1. Liu, Y., Fan, M., Zhang, S., Sheng, X. & Yao, J. Basic amino acid induced isomerization of a spiropyran: towards visual recognition of basic amino acids in water. *New J. Chem.,* **2007**, 31, 1878–1881.
2. Murugan, N. A.; Chakrabarti, S.; Agren, H. *Solid-Phase Peptide Synthesis*; 2011; Vol. 289.