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Electronic Supplementary Information

Light-controlled out-of-equilibrium assembly of cyclodextrins in an enzyme-mediated dynamic system

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Acknowledgements

We are grateful for the kind gift of the CGTase enzyme isolated from *Bacillus macerans* provided by Amano Enzyme Inc., Nagoya, Japan. We thank the Villum Foundation and Carlsberg Foundation for financial support.

S1. General experimental details and instrumental setup

S1.1 Materials

α-Cyclodextrin and all photoswitches except **3**, **4**, and **S4** were obtained from commercial suppliers and used as received. Photoswitches **3**, **4** and **S4** were obtained by acetylation of the commercially available non-acetylated analogs **S1** – **S3**. The synthetic procedures for **3** and **4** are reported elsewhere in this supporting information. **S4** was prepared according to a previously reported procedure and the isolated compound conformed to literature data.[S1] HPLC grade solvents or better as supplied from commercial sources were used for both synthesis and chromatographic analysis unless otherwise specified. High purity water was obtained by filtering deionised water through a commercial water purification system (Merck Millipore Synergy UV). Colorless Corning CoStar 0.65 mL centrifuge tubes were used for sample preparation (heating/acidification and centrifugation), while colorless 2 mL glass vials with PTFE-lined screw-cap septa with a 0.2 mL glass insert were used for short term sample storage and injection on chromatographic equipment. A stock solution of CGTase derived from *Bacillus macerans* was kindly gifted to our group by Amano Enzyme, Inc., Nagoya, Japan. It was used as supplied and stored at 5 °C.

S1.2 Instruments

Chromatographic analyses were performed on a Thermo Scientific Dionex UltiMate 3000 chromatography system with a Waters Acquity UPLC BEH Amide 1.7 µm 2.1 x 150 mm column maintained at 30 °C. The system was equipped with an autosampler which was maintained at 20 °C. Injection volumes were typically in the 1.0 – 10.0 µL range, depending on analyte concentrations. For detection, the chromatographic system was connected to an Agilent Technologies 1260 Infinity ELSD, operating at evaporator and nebulizer temperatures of 90 and 70 °C, respectively, and an N_2 gas flow of 1.0 L/min. NMR spectroscopy was performed on a Bruker 400 MHz instrument. High-resolution mass spectrometry (HRMS) were performed by the mass spectrometry facility at Department of Chemistry, University of Copenhagen. Melting points were measured on a Stuart SMP 30 melting point apparatus and are uncorrected.

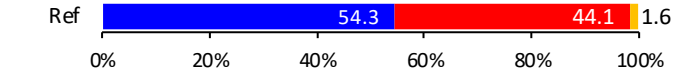
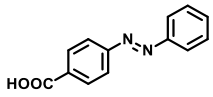
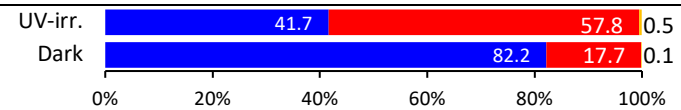
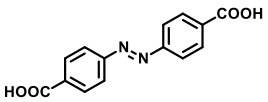
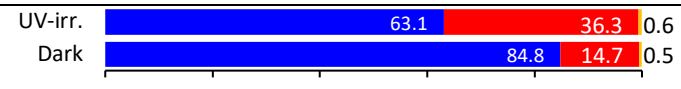
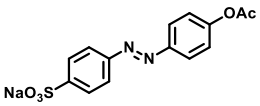
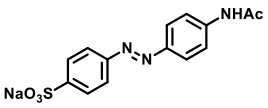
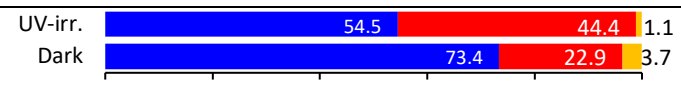
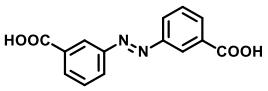
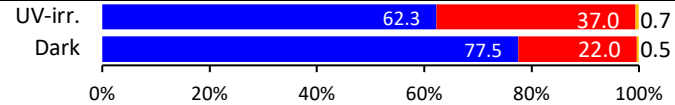
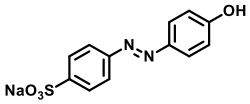
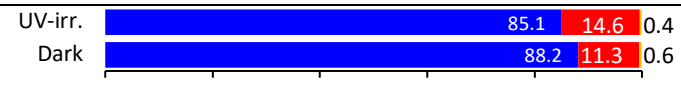
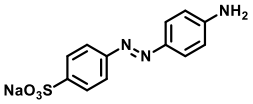
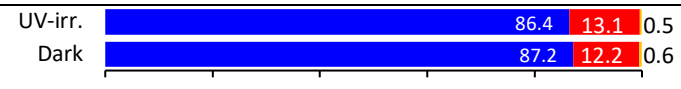
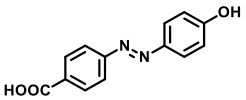
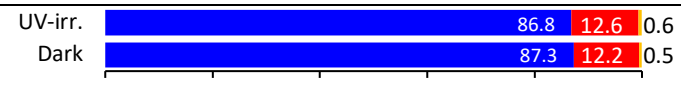
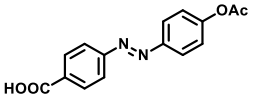
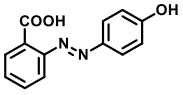
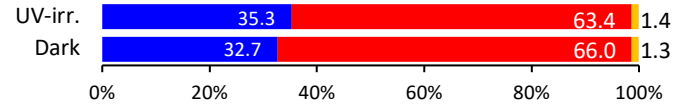
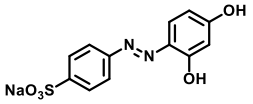
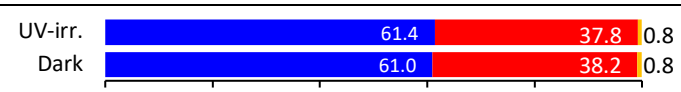
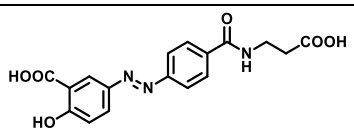
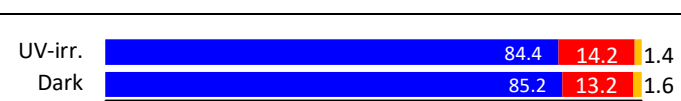
S2. Procedure for setting up and analysing CGTase-mediated dynamic systems

Solution of α-CD (2.0 mg/mL) with the desired template **1** – **S7** (2.0 mM) in sodium phosphate buffer (50 mM) at pH 7.5 was prepared. All reactions were set up in clear glass 2 mL vials, and they were started by adding CGTase stock solution (5 % (v/v)). For reactions under UV irradiation, the vials were installed in a specially fitted reaction chamber irradiated by a laboratory UV lamp (nominal 365 nm, 4 W, tubular 6 inch UV lamp). For consistency, the vials were placed at the exact same distance from the lamp each time, and, if more than one reaction was irradiated at the same time, the vials were spaced apart to ensure equal irradiation. For reactions under irradiation by blue light, a commercial mounted light-emitting diode (LED) array was used (Kessil A160WE Tuna Blue). The array consists of blue LEDs and broad spectrum white light LEDs. The lamp was turned to the most blue setting, in which only the blue LEDs are emitting, and it was used at the lowest possible intensity. Fan-assisted cooling ensured the enzymatic reactions stayed below 32 °C. For reactions in the dark, the vials were wrapped in aluminium foil prior to enzyme addition.

The enzymatic reaction was stopped by rapidly heating an aliquot (typically 30 – 60 µL) in a closed centrifuge tube to 100 °C by submerging it in a boiling water bath. It was kept in the water bath for at least 10 minutes to ensure full denaturisation of the enzyme. After cooling to room temperature, three volumes of acetonitrile were added and the denatured enzyme along with precipitated salts were removed by centrifugation at 10,000 RPM for ten minutes. Alternatively, the reaction was stopped by rapid four-fold dilution of an aliquot of the reaction (typically 10 – 60 µL) in acetonitrile with 1 % trifluoroacetic acid before centrifugation. The centrifugation step was performed only to improve chromatographic column life times (by preventing blockage from build-up of insoluble material over many injections), and comparisons to non-centrifuged samples showed no change in the concentrations of the analytes of interest in this study (i.e. the sugars). For storage (at 20 °C) and injection on chromatographic equipment, samples were transferred to 2 mL glass vials (often with 0.2 mL glass inserts) and capped with screw caps equipped with PTFE lined rubber septa.

S3. Photoswitches screened as templates for dynamic enzymatic reactions

Table S1 Structure of photoswitches screened in dynamic libraries and the CD composition of those libraries after 2 hours, both upon UV irradiation (365 nm) and when kept in the dark

#	Structure	**CD composition after 2h** (in % of total CD concentration) (■ α-CD, ■ β-CD, ■ γ-CD)
Ref	(no photoswitch added)	
1		
2		
3		
4		
5		
S1		
S2		
S3		
S4		(not soluble under the conditions of the experiment)
S5		
S6	 (Trivial name: Trapaeolin 0)	
S7	 (Trivial name: Balsalazide)	

S4. Chromatograms from enzymatic reactions with photoswitches 1 – 5 with/without UV-irradiation

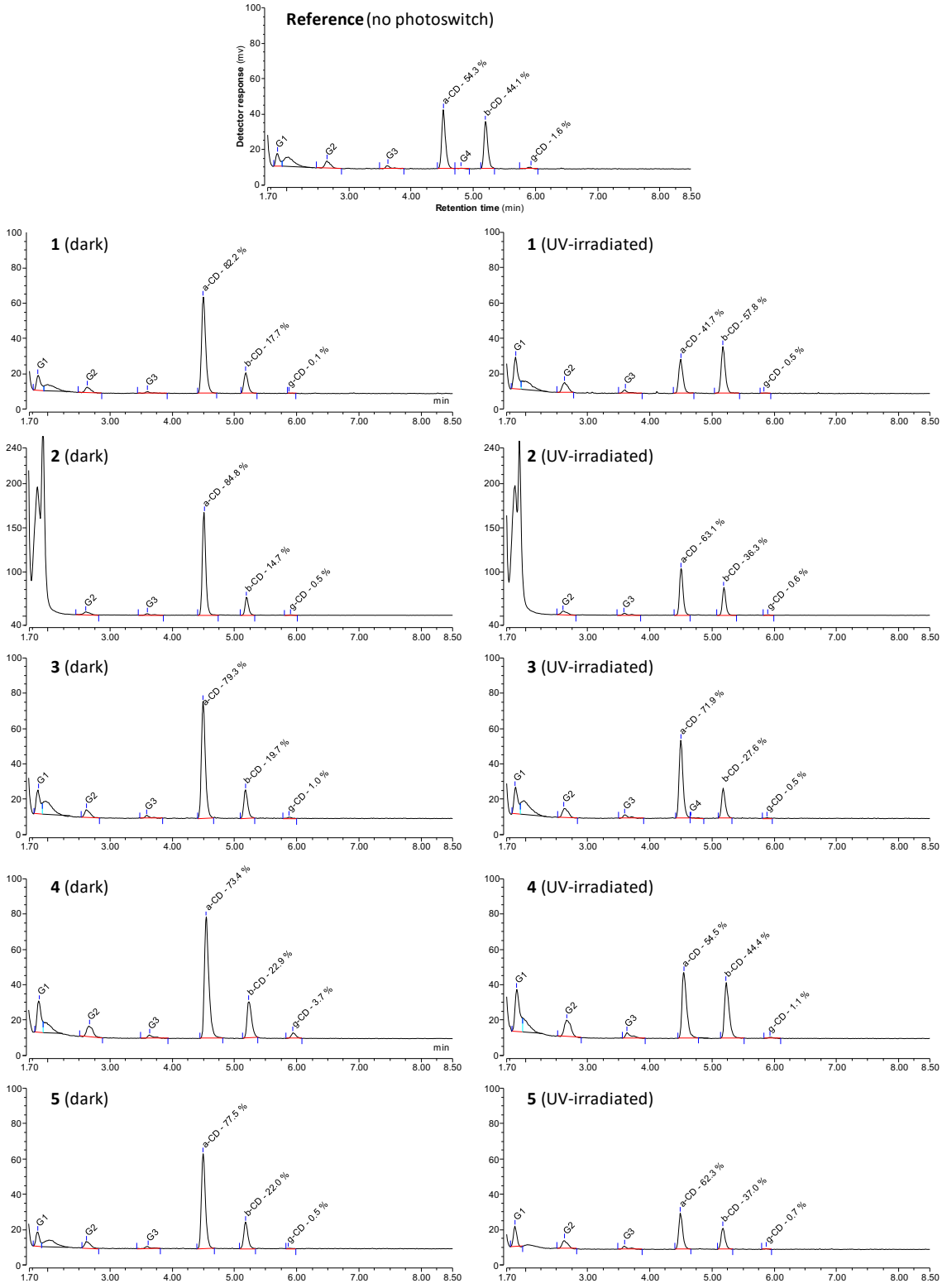


Figure S1. Chromatograms (HPLC-ELS) of enzymatic reactions after 120 minutes with photoswitches **1** – **5** in the dark (left) and with UV-irradiation (365 nm, 1 h prior to addition of enzyme and throughout the two-hour experiment) (right), as well as a reference library with no photoswitch (top). Conditions as in figure 2 of the manuscript. **G1** = glucose, **G2** = maltose, **G3** = maltotriose, **G4** = maltotetraose.

S5. UV-vis spectra of photoswitches 1 – 5 in phosphate buffered water with/without UV irradiation

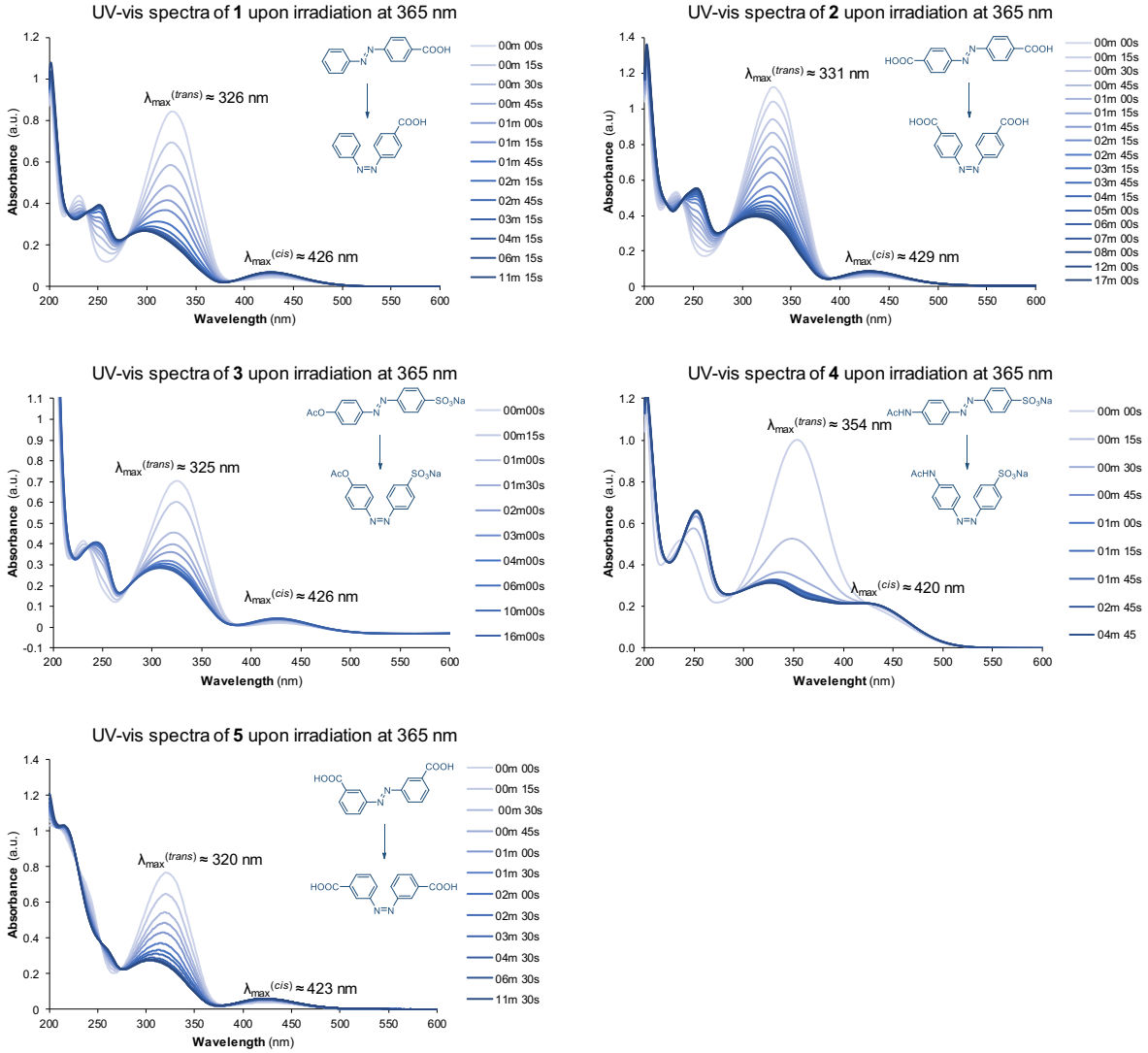
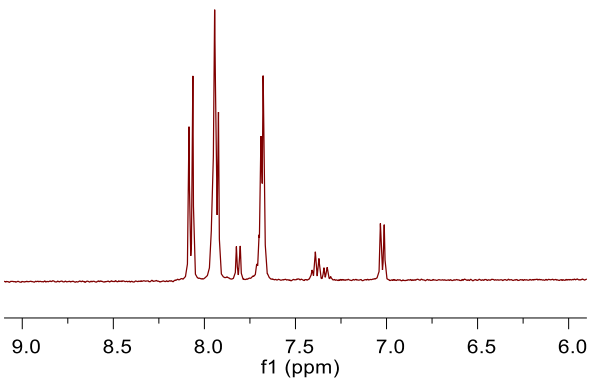


Figure S2. UV-vis absorption spectra from 200 to 600 nm of photoswitches **1** – **5** (40 µM) in sodium phosphate buffered (50 mM, pH 7.5) water at room temperature. Solutions kept in ambient light conditions were transferred to a cuvette and the first spectrum was measured. The cuvette was transferred to the same irradiation source used for the enzymatic reactions (i.e. irradiation at 365 nm) (section S2) for the indicated amount of accumulated time and then another spectrum was measured. This procedure was followed until no further changes in the spectrum were observed.

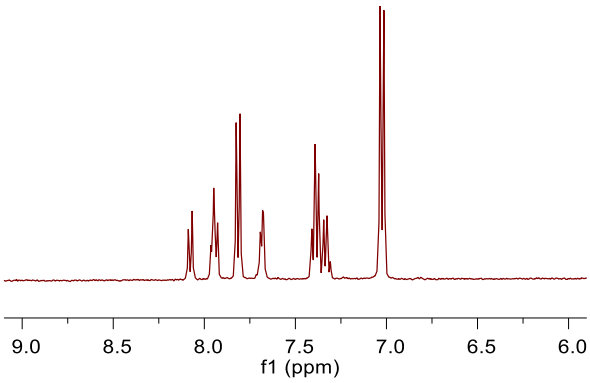
S6. ^{1}H NMR spectra of photoswitches 1 – 5 in phosphate buffered water with/without UV irradiation

Solutions of **1** – **5** (1.00 mM) in sodium phosphate buffered (50 mM, pH 7.5) D_2O were prepared. An aliquot of each was exposed to UV irradiation at 365 nm for 60 minutes under the same conditions as the enzymatic reaction (section S2) before it was transferred to an NMR tube wrapped in aluminium foil (to prevent isomerization promoted by ambient light). The sample was kept in the dark until it was measured. *Trans* and *cis* isomer signals were identified by comparison with non-irradiated samples, and their relative concentrations were evaluated by integrations of appropriate signals that had no or very little interference from overlapping signals.

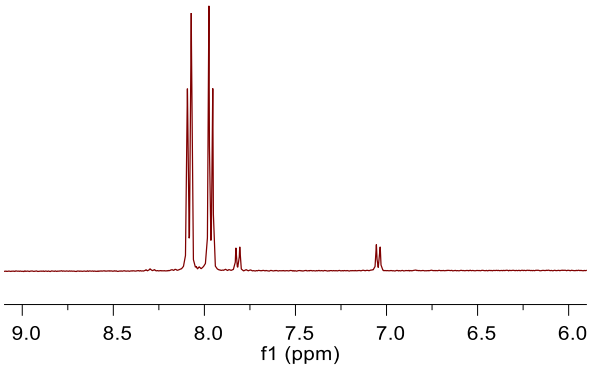
1 (1 mM, no irradiation):



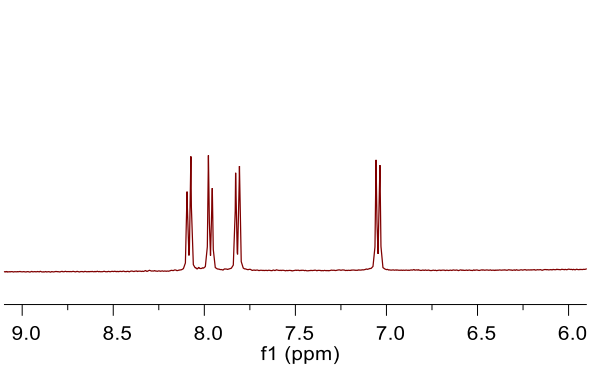
1 (1 mM, irradiated at 365 nm for 1h):



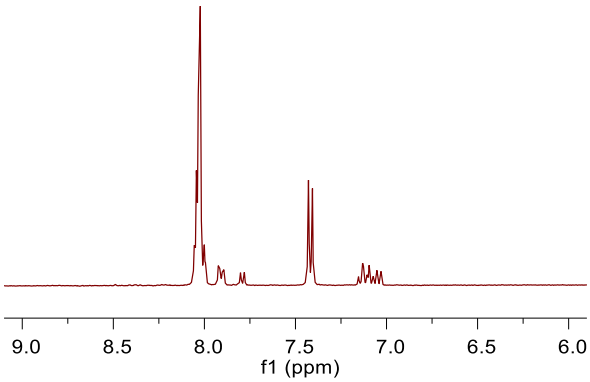
2 (1 mM, no irradiation):



2 (1 mM, irradiated at 365 nm for 1h):



3 (1 mM, no irradiation):



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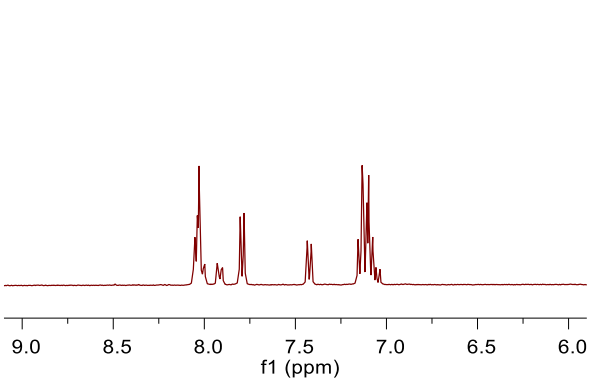
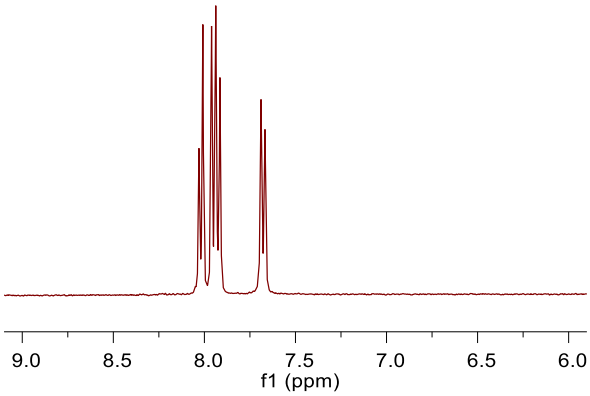
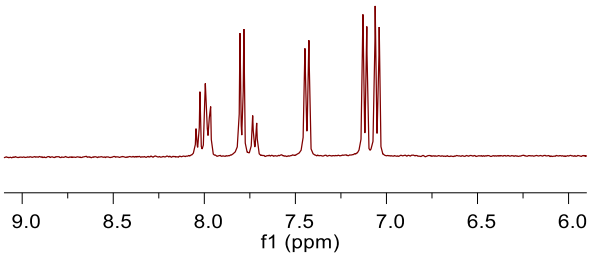


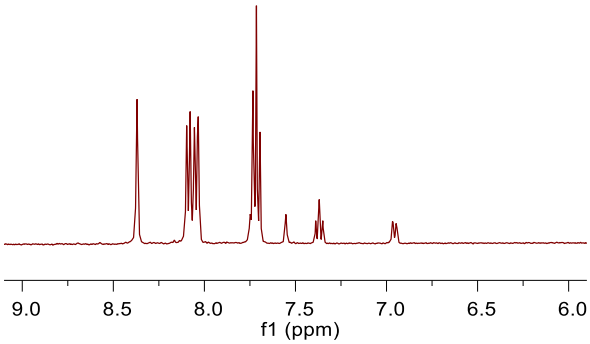
4 (1 mM, no irradiation):



4 (1 mM, irradiated at 365 nm for 1h):



5 (1 mM, no irradiation):



5 (1 mM, irradiated at 365 nm for 1h):

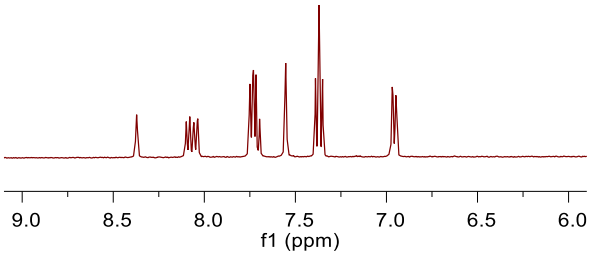


Figure S3 (on this and the preceding page). ^{1}H NMR spectra of photoswitches **1** – **5** (1.00 mM) in sodium phosphate buffered (50 mM, pH 7.5) D_2O in ambient light conditions (left) or after irradiation at 365 nm for 60 minutes (right). Irradiation was performed under the same conditions as for the enzymatic reactions (section S2).

S7. ^{1}H NMR titrations of *trans*-1 with α-CD and β-CD in phosphate buffered D_2O

Stock solutions of **1** (1.00 mM) and **1**+α-CD (1.00+10.0 mM) or **1**+β-CD (1.00+10.0 mM) in sodium phosphate buffered (50 mM, pH 7.5) D_2O were mixed in appropriate proportions to produce the eleven concentrations of titrants noted below (0 – 10.0 mM). The sealed NMR tubes were then heated to 80 °C in the dark for two days to ensure **1** existed only in the *trans* isomer. The tubes were allowed to cool to room temperature in the dark and were then analysed by ^{1}H NMR spectroscopy, making sure to minimize exposure to ambient light. Measurements were performed at 298 K. The change in the chemical shift, Δδ, was monitored for the sharpest peak of *trans*-**1** in the resulting spectra, and the values were fitted non-linearly to a 1:1 binding model to determine the binding affinity, K_a, and the chemical shift difference between the 1:1 complex and the free photoswitch, $\Delta\delta_{max}$, for *trans*-**1**·α-CD and *trans*-**1**·β-CD, respectively.

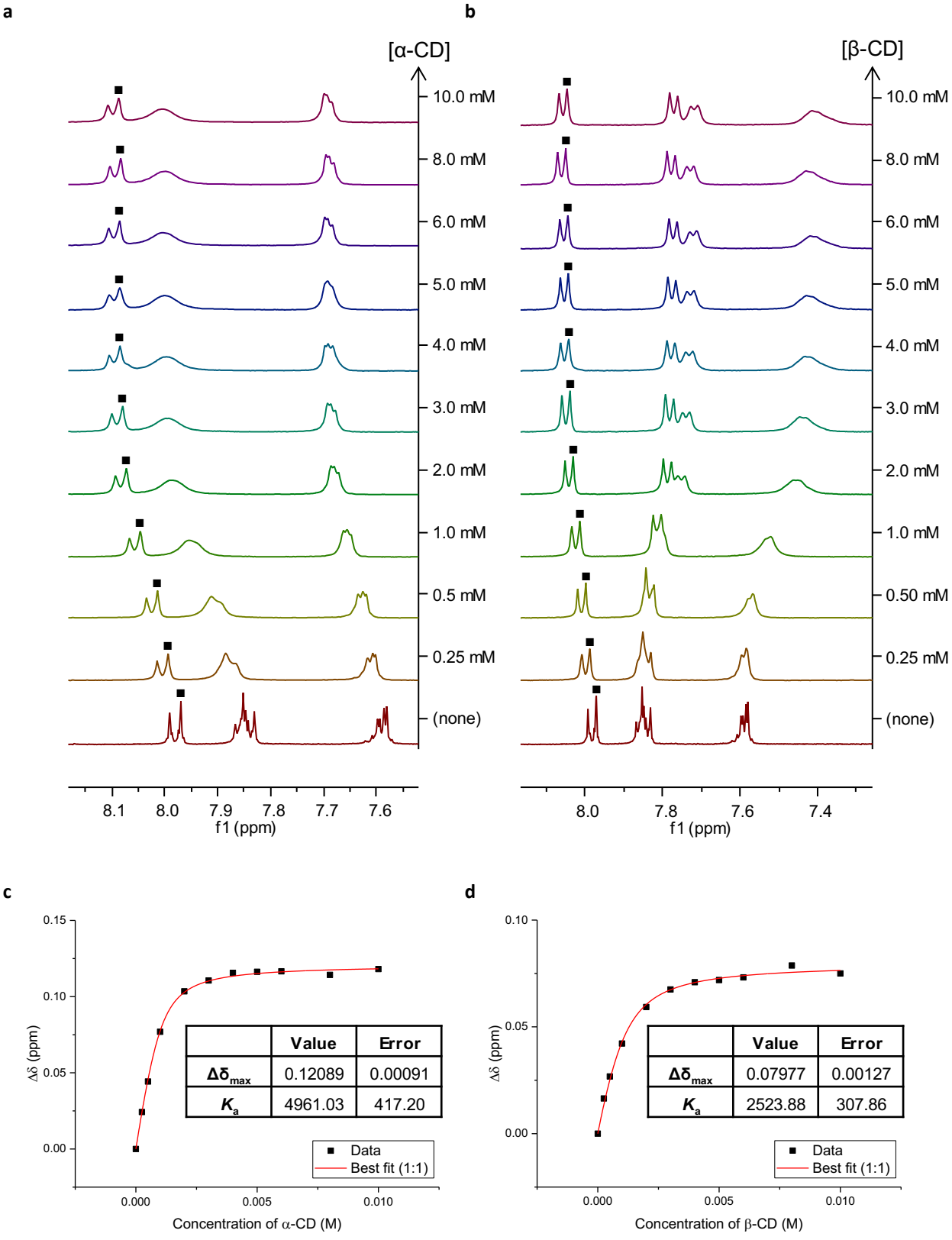


Figure S4 (on this and the preceding page). a,b) ^{1}H NMR spectra of *trans*-**1** in presence of increasing amounts of α-CD and β-CD, respectively. The monitored peak is identified with a black square (■). c,d) Plot of the change in chemical shift, $\Delta\delta$, of the monitored peak and the best fit to a 1:1 binding isotherm for α-CD and β-CD, respectively. Tabulated are the best-fit values determined for the maximum change in the chemical shift, $\Delta\delta_{max}$, and the binding constant, K_a, along with their respective fitting errors.

S8. Determination of binding constants for *cis*-1·α-CD and *cis*-1·β-CD by competitive ^{1}H NMR titration

Stock solutions of **1** (1.00 mM) and **1**+α-CD (1.00+5.00 mM) or **1**+β-CD (1.00+10.0 mM) in sodium phosphate buffered (50 mM, pH 7.5) D_2O were mixed in appropriate proportions to produce the thirteen concentrations of titrants noted below (0 – 5.00 mM or 0 – 10.0 mM, respectively). The tubes were left in ambient light and temperature and measured three times each over several days (day 4, 7, and 12 after mixing). Figure S5 shows representative spectra measured on day 7 after mixing. The change in the chemical shift of *trans*-**1**, $\Delta\delta_{trans\text{-}\mathbf{1}}$, was plotted against the corresponding changes in the chemical shift of *cis*-**1**, $\Delta\delta_{cis\text{-}\mathbf{1}}$. Using the binding constants and $\Delta\delta_{max}$ for *trans*-**1**·α-CD and *trans*-**1**·β-CD determined above, the data was fitted non-linearly to the equation stated in the manuscript.[14] For reporting K_a and $\Delta\delta_{max}$ values for *cis*-**1**, the fitting errors were compounded with the errors on the values determined for *trans*-**1**, since the latter values were used to determine the former.

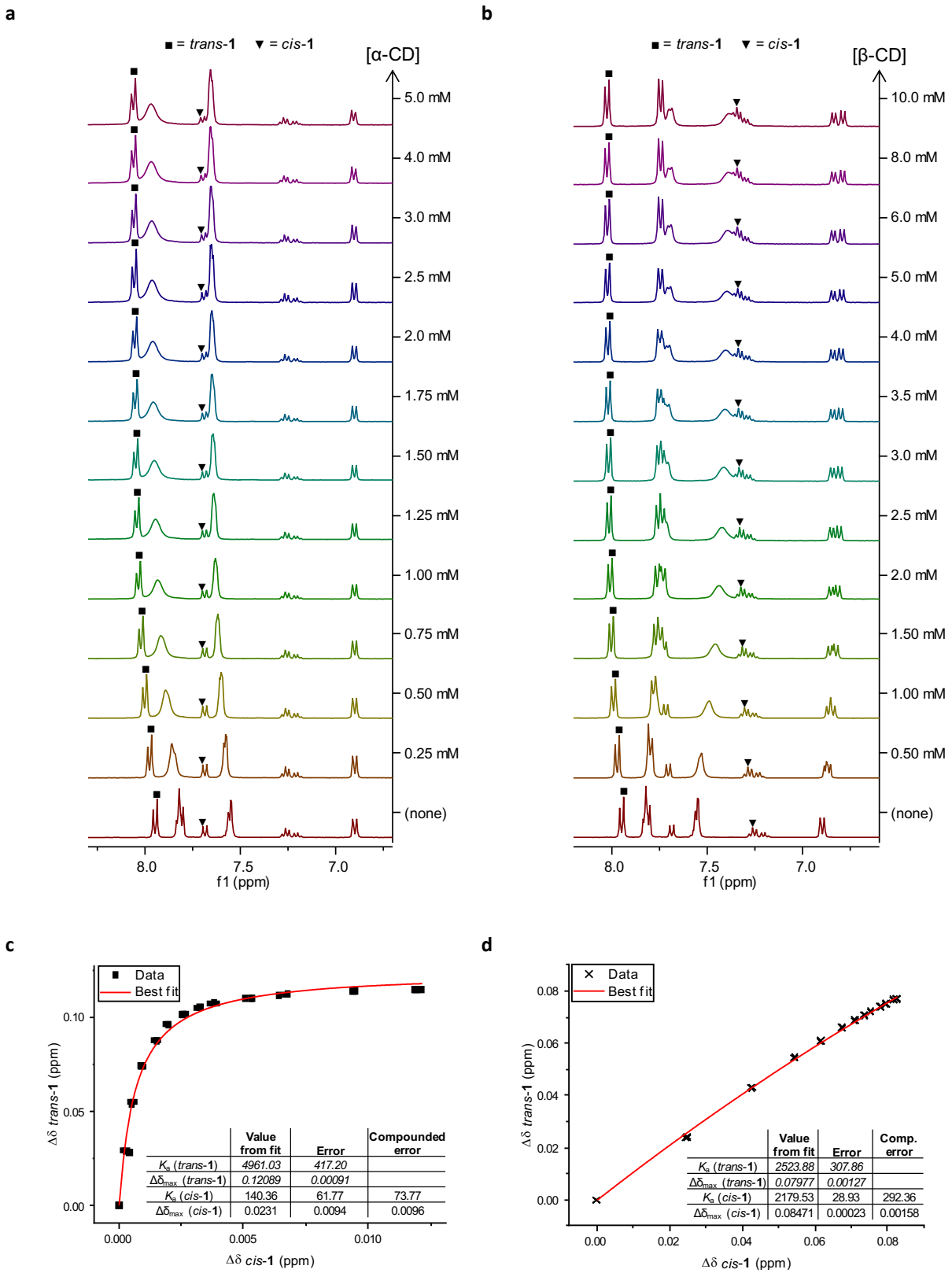


Figure S5. a,b) 1H NMR spectra of *cis*- and *trans*-**1** in presence of increasing amounts of α-CD and β-CD, respectively. The monitored peaks are identified with a black square (■) for *trans*-**1** and black triangle (▼) for *cis*-**1**. c,d) Plot of the change in chemical shift of *trans*-**1**, $\Delta\delta_{trans\text{-}1}$, vs the concurrent change in the chemical shift of *cis*-**1**, $\Delta\delta_{cis\text{-}1}$, and the best fit to the equation listed in the manuscript for both α-CD and β-CD, respectively. Tabulated are the best-fit values and errors determined for *trans*-**1** in the titration above (section S7), which were put in as known constants in this fit, along with the values determined for *cis*-**1** in this fit. Listed are both the errors on this fit, and the compounded errors that were calculated based on the errors on both fits.

S9. CD distribution and evolution of dynamic CD systems under different light conditions

Reference libraries

The CGTase-catalysed interconversion of cyclodextrins in the presence of photoswitch **1** was monitored over time in the dark and another under constant UV irradiation to provide reference experiments for the reactions depicted in Figure 3a,b of the main article (Figure S6). These results showed that it takes ca. 30-40 minutes to reach equilibrium after addition of CGTase Furthermore, these reactions confirm the trend that a higher degree of background hydrolysis is seen under UV irradiation compared to reactions in the dark or under blue light irradiation.

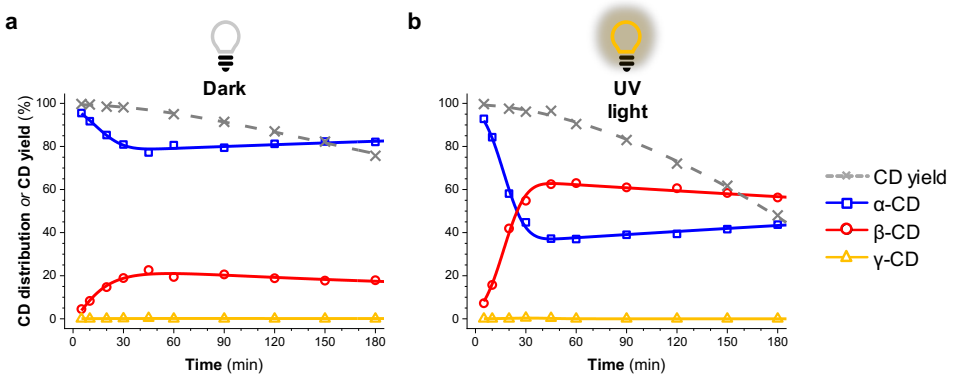


Figure S6. CD distribution over time when CGTase acts on α-CD under different light conditions. a) Kept in the dark. b) Under constant UV irradiation (365 nm). Conditions as in Figure 3 of the manuscript.

UV/blue light irradiation cycles

To assess how long the dynamic reaction takes to switch to the new equilibrium distribution upon changing the irradiation from blue light to UV light, a single reaction was monitored over time upon performing such a switch (Figures S7). The results showed that it takes ca. 20 minutes for the dynamic enzymatic system to reach the new equilibrium distribution upon switching from blue light to UV light irradiation, as opposed to the only ca. 15 minutes needed when switching the other way (see Figure 3b of the manuscript).

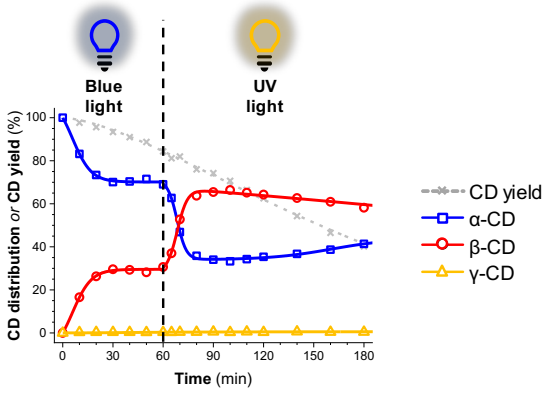


Figure S7. CD distribution and CD yield over when CGTase acts on α-CD initially under blue light irradiation (460 nm) for 60 minutes and then UV irradiation (365 nm). Conditions as in Figure 3 of the manuscript.

Based on these results, it was decided to use a 20-minute UV irradiation time and a 15 minute blue light irradiation time for consecutive cycles of photoswitching, except that 30 mins is required to reach the initial equilibrium in the first step of the first cycle (Table S2).

A total of twelve cycles were performed, showing a high degree of switching during all cycles (Figure S8). Background hydrolysis is significant over the course of the full 9-hour experiment. This is reflected in the continuously increasing proportion of α-CD. Figure S9 the relative concentrations of all species present (α-, β-, γ-CD and **G1**-**G8**) in the dynamic enzymatic system during 12 cycles of photoswitching.

Table S2. Irradiation times during the experiments to switch the dynamic system back and forth between UV- and blue light-induced equilibrium distributions.

Cycle	Irradiation source	Irradiation time	Total reaction time	Notes
Cycle 1	UV (365 nm) Blue light (460 nm)	30 min 15 min	30 min 45 min	To allow initial equilibrium to be reached.
Cycle 2	UV (365 nm) Blue light (460 nm)	20 min 15 min	65 min 80 min	Irradiation times following same pattern (20 + 15 min) from this cycle onwards.
Cycle 3	UV (365 nm) Blue light (460 nm)	20 min 15 min	100 min 115 min	
Cycle 4	UV (365 nm) Blue light (460 nm)	20 min 15 min	135 min 150 min	
Cycle 5	UV (365 nm) Blue light (460 nm)	20 min 15 min	170 min 185 min	
Cycle 6	UV (365 nm) Blue light (460 nm)	20 min 15 min	205 min 220 min	
Cycle 7	UV (365 nm) Blue light (460 nm)	20 min 15 min	240 min 255 min	
Cycle 8	UV (365 nm) Blue light (460 nm)	20 min 15 min	275 min 290 min	
Cycle 9	UV (365 nm) Blue light (460 nm)	20 min 15 min	310 min 325 min	
Cycle 10	UV (365 nm) Blue light (460 nm)	20 min 15 min	345 min 360 min	
Cycle 11	UV (365 nm) Blue light (460 nm)	20 min 15 min	380 min 395 min	
Cycle 12	UV (365 nm) Blue light (460 nm)	20 min 15 min	415 min 430 min	

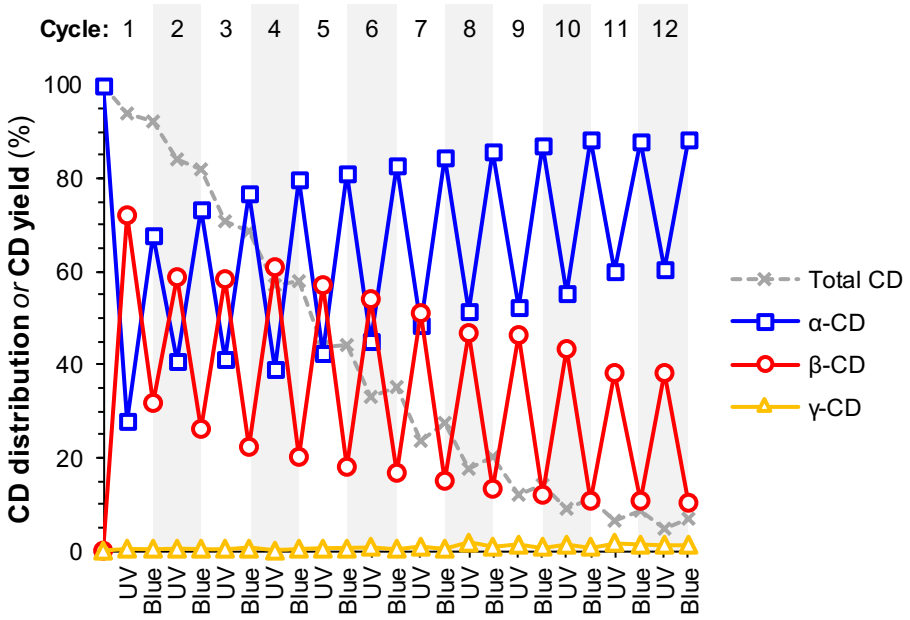


Figure S8. CD distribution (out of all CDs) and CD yield (% by weight of all sugars) at the end of each step in the twelve two-step UV/blue light cycles performed as outlined in Table S2. Conditions otherwise as in Figure 3 of the manuscript.

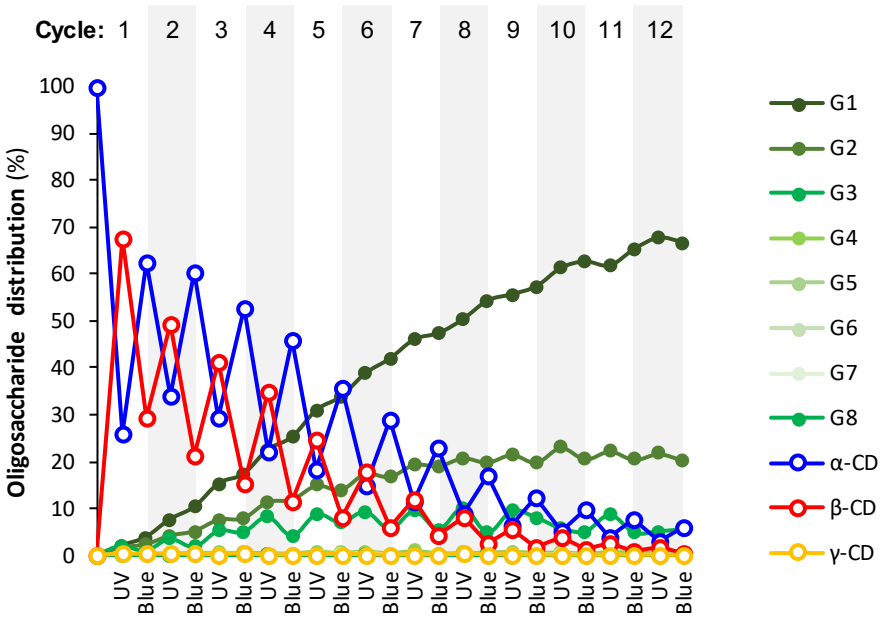
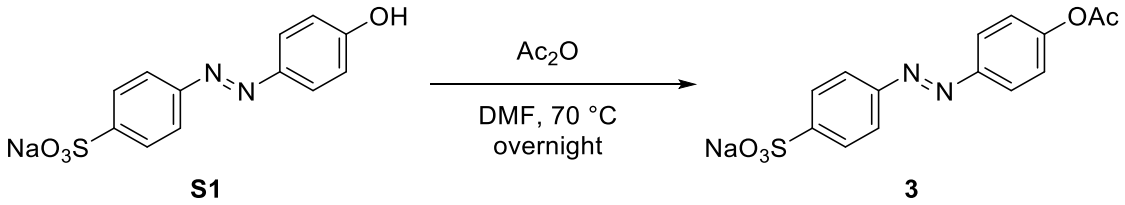


Figure S9. Distribution of CDs and linear maltooligosaccharides (G1-G8) during the consecutive UV/blue light cycles. The distributions are based on concentrations by weight, and thus represents how much of the total glucose content makes up each of the species present.

S10. Synthetic procedures

S10.1 Synthesis of photoswitch **3**



S1 **3**

This procedure was modified from the literature procedure for the corresponding sulfonic acid.[S2] **S1** (52 mg, 0.17 mmol) was suspended in DMF/acetic anhydride (1:6, 14 mL) and stirred at 70 °C overnight. After cooling the mixture to room temperature, the precipitate was isolated by suction filtration and allowed to air dry. Yield: 17 mg (29 %).

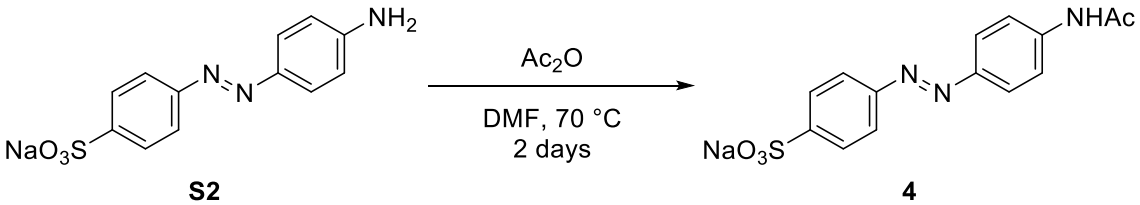
Melting point: Decomposes above 300 °C.

HRMS (m/z): Calculated for [$C_{14}H_{11}N_2O_5S^-$]: 319.0394; found: 319.0395.

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.96 (d, *J* = 8.8 Hz, 2H), 7.86 (d, *J* = 8.5 Hz, 2H), 7.80 (d, *J* = 8.5 Hz, 2H), 7.36 (d, *J* = 8.8 Hz, 2H), 2.30 (s, 3H) ppm.

^{13}C NMR (101 MHz, DMSO-d_6) δ 169.38, 153.07, 151.88, 150.59, 149.78, 127.00, 124.14, 123.23, 122.44, 21.12 ppm.

S10.2 Synthesis of photoswitch **4**



S2 **4**

S2 (1.04 g, 34.8 mmol) was suspended in DMF/acetic anhydride (1:6, 140 mL) and stirred at 70 °C for 2 days. Large insoluble clusters of solids were removed by decantation and the remaining precipitate was isolated by suction filtration and washed with cold ethanol. Yield: 0.711 g (60 %).

Melting point: Decomposes above 350 °C.

HRMS (m/z): Calculated for [$C_{14}H_{12}N_3O_4S^-$]: 318.0554; found: 318.0555.

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.32 (s, 1H), 7.89 (d, *J* = 8.9 Hz, 2H), 7.84 – 7.75 (m, 6H), 2.10 (s, 3H) ppm.

^{13}C NMR (101 MHz, DMSO-d_6) δ 168.94, 151.77, 150.36, 147.47, 142.55, 126.74, 123.82, 121.90, 119.16, 24.22 ppm.

S11. NMR spectra of newly synthesized compounds

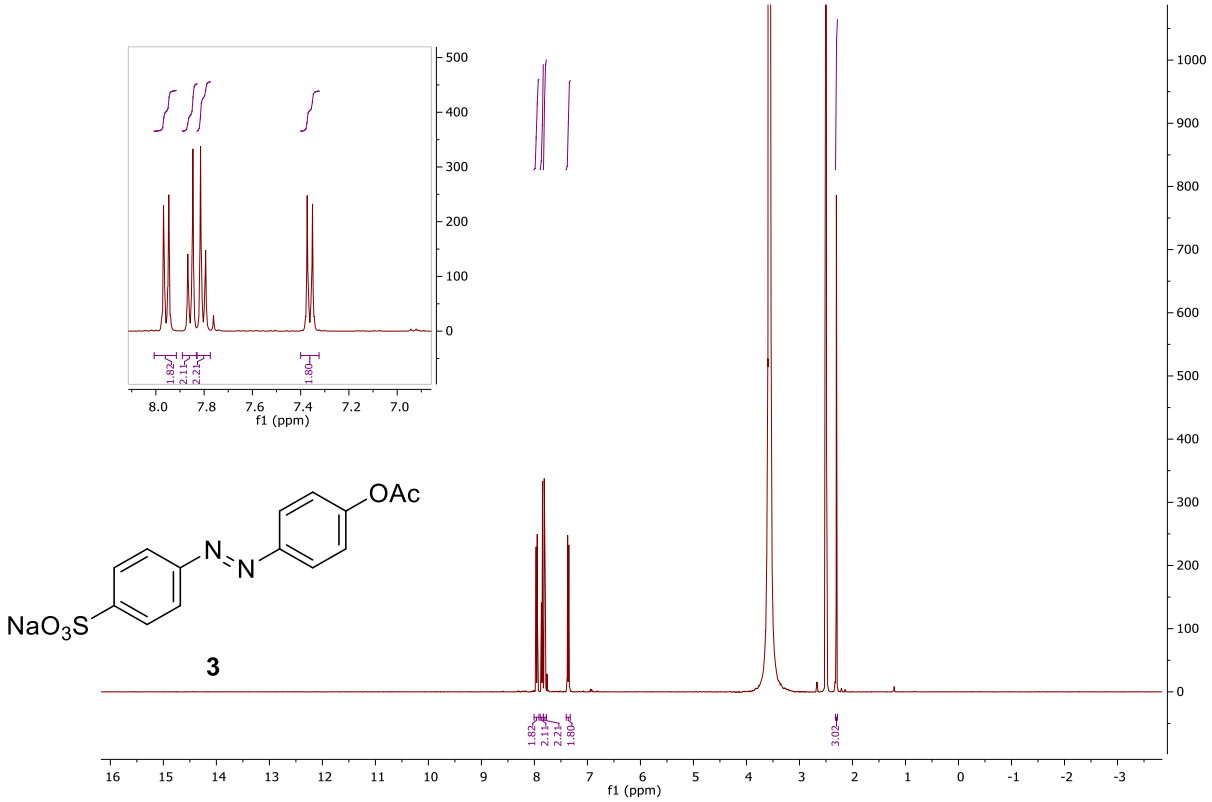


Figure S10: ^{1}H NMR spectrum of **3** in DMSO-d_6 with expansion of the aromatic region (frame in upper left corner), showing presence of a small amount of the *cis* isomer.

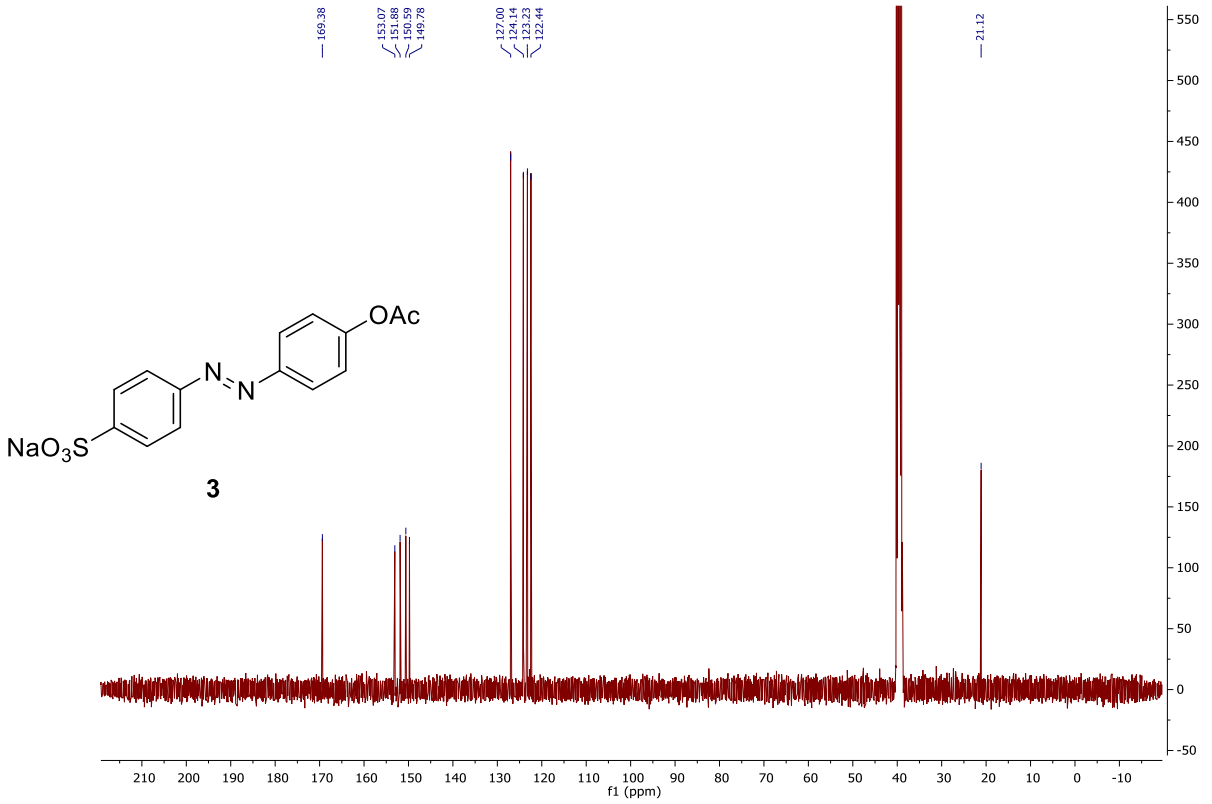


Figure S11: ^{13}C NMR spectrum of **3** in DMSO-d_6.

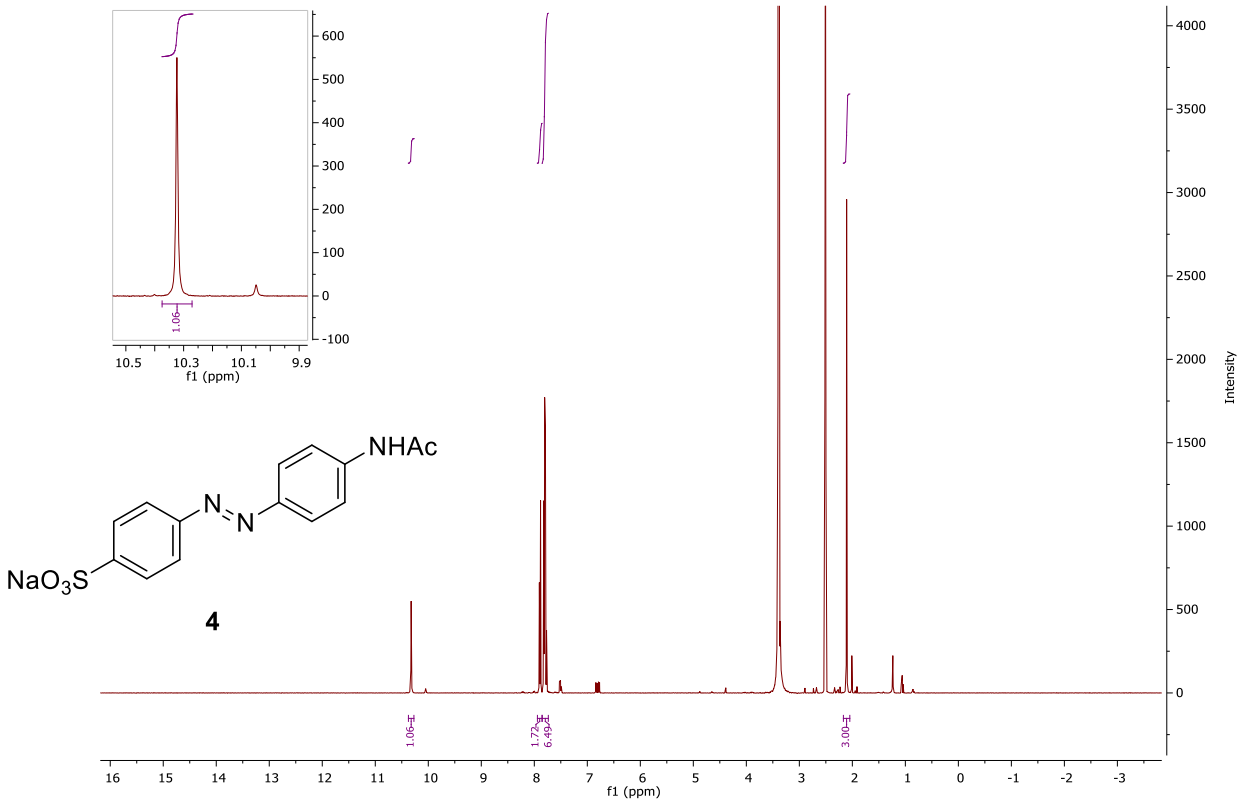


Figure S12: ^{1}H NMR spectrum of **4** in DMSO-d_6. There is a small amount of the *cis* isomer present, as evidenced by the expansion of NH signal (frame in upper left).

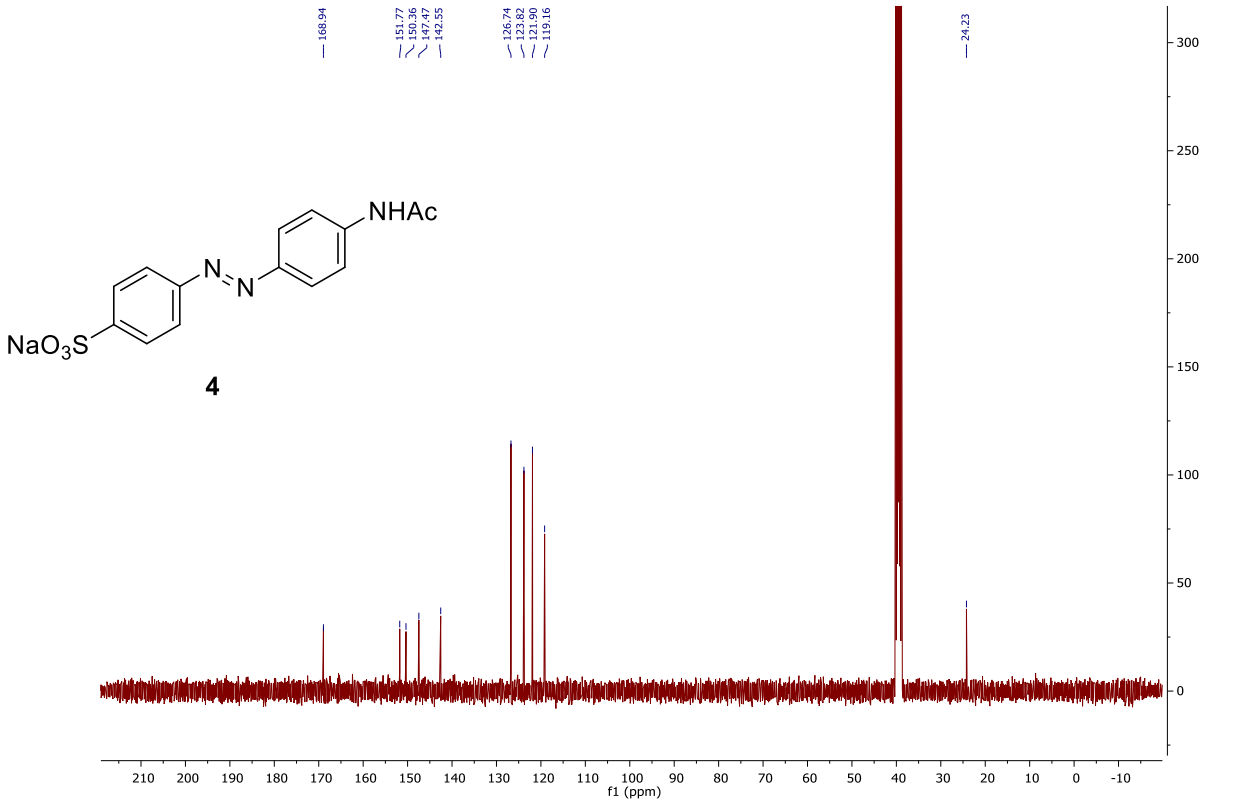


Figure S13: ^{13}C NMR spectrum of **4** in DMSO-d_6.

S12. Supporting References

(1) Oertel, U.; Mart, H.; Komber, H.; Böhme, F. *Opt. Mater.* **2009**, *32*, 54-61.

(2) Suh, J.; Kwon, W. J. *Bioorg. Chem.* **1998**, *26*, 103-107.