

Supporting Information

Two-photon-Induced [2+2] Cycloaddition of Bis-thymines: A Biocompatible and Reversible Approach

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Experimental procedures

Materials

Thymine based compounds were synthesized and characterized by the group of Prof. K. Saito following a previously described protocol.^[1] One-photon induced polymerization and depolymerization was conducted by Saito et al., using a previously reported procedure.^[1] Here, the monomer powder was spread into a thin layer (approx. 1 mm thick) on a Pyrex petri dish and irradiated with 302 nm light (850 J.cm^{-2}) (Ultraviolet Products CL1000M UV-crosslinker lamp) to induce polymerization. The photosynthesized polymer was then converted into the starting monomer upon irradiation with $< 240 \text{ nm}$ light (850 J.cm^{-2}) (Ultraviolet Products CL1000M UV-crosslinker lamp).

Sample preparation

The monomer powder was dissolved in a 50/50 (v/v) mixture of Acetonitrile/ H_2O (10 mg.mL^{-1} , $21 \mu\text{M}$). The solution was vortexed to ensure that all monomer was dissolved and $70 \mu\text{L}$ were dropcasted on a clean glass coverslip. Solvent was allowed to evaporate overnight to yield white crystals, attached to the glass coverslip (Figure S1). One-photon synthesized polymer was either dissolved in a 50/50 (v/v) mixture of Chloroform/ H_2O (10 mg.mL^{-1}) to yield a film-like morphology or in a 50/50 (v/v) mixture of Acetonitrile/ H_2O (10 mg.mL^{-1}) to yield crystal-like morphology. Both solutions were then dropcasted onto a glass coverslip following the same procedure described for the monomer. After solvent evaporation film and crystal structures appeared (Figure S3).

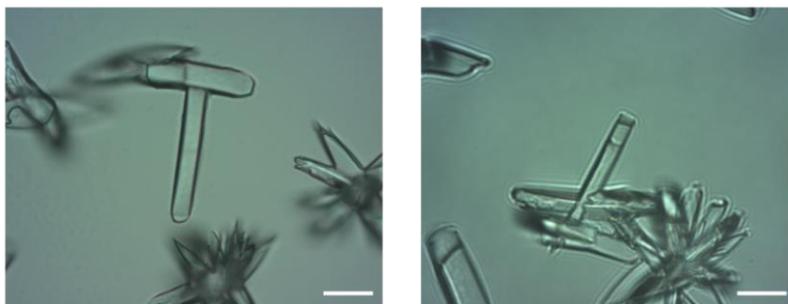


Figure S1. Transmission images of the monomer crystals formed after solvent evaporation. The images have been recorded from two different areas in the glass coverslip. Scale bar: $10 \mu\text{m}$.

Raman spectroscopy

Raman measurements were conducted using an inverted optical microscope (Ti-U, Nikon) equipped with a piezoelectric stage (P517.3CL, Physik Instrument). Continuous wave He-Ne laser light (632.8 nm) was reflected by a dichroic mirror (Z633RDC, Chroma) and was then focused to the sample by an objective lens (Plan Apo 60x, N.A. 0.95 or Plan Fluor 40x, N.A. 0.60, Nikon). Circular polarization at the sample was achieved by tuning half-wavelength ($\lambda/2$) and quarter-wavelength ($\lambda/4$) waveplates. The Raman signals were collected by a spectrometer (iHR 320, Horiba) equipped with a cooled electron multiplying charge-coupled device (CCD) camera (Newton 920, Andor).

Two-photon reaction

Two-photon experiments were performed on the same set-up used for Raman spectroscopy. A glass coverslip containing either monomer or polymer was placed onto the piezoelectric stage of the microscope. A Ti:sapphire femtosecond laser (MaiTai, Spectra-Physics, 820 nm, 120 fs, 80 MHz) coupled with an optical parametric oscillator (OPO) (Inspire, Spectra-Physics) was used to produce femtosecond pulses (80 MHz, 200 fs, linearly polarized) to induce two-photon reactions. The light was deflected by a dichroic mirror before it was focused on the sample by the same objective as used for Raman spectroscopy. Three different wavelengths were used, i.e. 600 nm for cycloaddition (1.6 mW), 450 nm for cycloreversion (0.3 mW) and 500 nm for obtaining the unknown compound (1 mW). For a typical experiment an irradiation time of 1 minute was used.

Pearson correlation coefficient (PCC)

The sample Pearson correlation coefficient (PCC) between two spectra was estimated according to the following equation:

$$r_{x,y} = \frac{\sum_{n=1}^N (x_n - \bar{x})(y_n - \bar{y})}{\sqrt{\sum_{n=1}^N (x_n - \bar{x})^2} \sqrt{\sum_{n=1}^N (y_n - \bar{y})^2}}$$

where N denotes the total number of considered Raman shifts, x_n and y_n represent the Raman intensities registered for the first and second spectrum, respectively, at the n -th Raman shift and \bar{x} and \bar{y} are the mean intensity values recorded for the first and second spectrum, respectively. $r_{x,y}$ can be regarded as a measure of the overall similarity between the two profiles under study. Even though there exist no widespread consensus on the interpretation of the PCC, the coefficients calculated in this work reflect relatively high correlations given the noise level of the measurements and the high amount of experimental factors influencing them. In order to obtain an unbiased estimation of the PCC, each pair of spectra was preliminarily baseline-corrected by Asymmetric Least Squares (AsLS).^[2]

Raman characterization and peak assignment

The two-photon reaction was investigated via Raman spectroscopy because of its unique fingerprinting capabilities, which provide chemical and structural information specific for each chemical species.^[2] Particularly, [2+2] cycloaddition reactions can be easily monitored via Raman spectroscopy as the disappearance of the aromatic moieties and the formation of the cyclobutane ring can be clearly distinguished in the Raman fingerprints.^{[3][4]}

On this basis, Raman fingerprints of the monomer and the polymer compounds obtained via one-photon process^[1] were collected and used as reference. A tentative assignment of the principal Raman bands has been carried out. As expected, the Raman spectra show characteristic fingerprints of the monomer and polymer structures, respectively (dashed vertical lines in Figure. S2). Specifically, the Raman spectrum of the monomer is characterized by the C=C double bond stretching at ~ 1656 cm^{-1} , pyridine ring stretching at ~ 1638 cm^{-1} and pyridine ring breathing at ~ 1067 cm^{-1} . In addition, other peaks associated with the aromatic moieties are present at ~ 447 cm^{-1} and ~ 463 cm^{-1} , arising from the N=C=C and at ~ 510 cm^{-1} and ~ 524 cm^{-1} , arising from the C-C=C.

The spectrum obtained from the polymer is characterized by the disappearance of the aromatic fingerprint and the emergence of the peaks at ~ 498 cm^{-1} , ~ 707 cm^{-1} and ~ 922 cm^{-1} , corresponding to the cyclobutane skeletal stretching mode.

Conformational changes in the aliphatic chains upon irradiation could also be observed in the spectra, particularly in the region between 1200 and 1500 cm^{-1} , corresponding to $-\text{CH}_2$, CH_3 and C-H in plane deformations. Additionally, the C=O stretching band at 1696 cm^{-1} shifts to 1714 cm^{-1} upon cycloaddition.

Such changes in the peaks assigned to the aliphatic chains are likely due to the structural rearrangements happening upon cycloaddition.

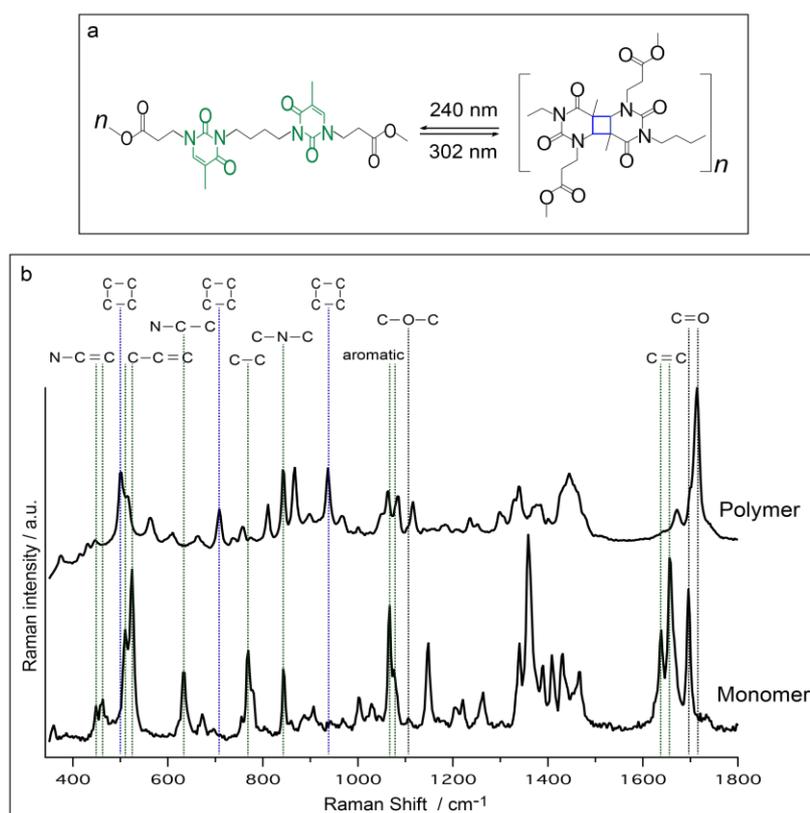


Figure. S2. Tentative assignment of the main Raman bands along with the monomer and polymer chemical structures, placed here for easier spectral interpretation. (a) Reaction scheme and chemical structures. The characteristic functional groups related to the monomer and polymer are highlighted in green and blue, respectively. (b) Raman spectra collected from the monomer and polymer samples obtained via one-photon reaction. Characteristic vibrational peaks are represented with dashed vertical lines. Molecular vibrations associated with the monomer are represented in green colour, whereas polymer related bands appear in blue. Chemical bonds in common with both monomer and polymer structures are illustrated in black.

Morphology dependence

To examine the influence of the polymer morphology on the Raman fingerprint, a small amount of one-photon induced polymer was dissolved in a 50/50 (v/v) mixture of Acetonitrile/H₂O to yield small crystal-like structures and in a 50/50 (v/v) mixture of Chloroform/H₂O to yield a film-like structure. The solutions were then dropcasted onto a glass coverslip, followed by solvent evaporation. Raman spectra of each morphology were measured (Figure. S3). Overall, the Raman spectra of both polymers show the same fingerprint, revealing only small differences related to a few peaks. The most notable difference being the band at 668 cm⁻¹, which is present in the film-like morphology, but absent in the crystal-like structure. In addition, the band at 937 cm⁻¹ appears only in the crystal-like polymer. In spite of this, both Raman spectra reveal a good fingerprint match. These results confirm our hypothesis that the small spectral variations observed between the two-photon and one-photon generated polymers originate from morphology differences.

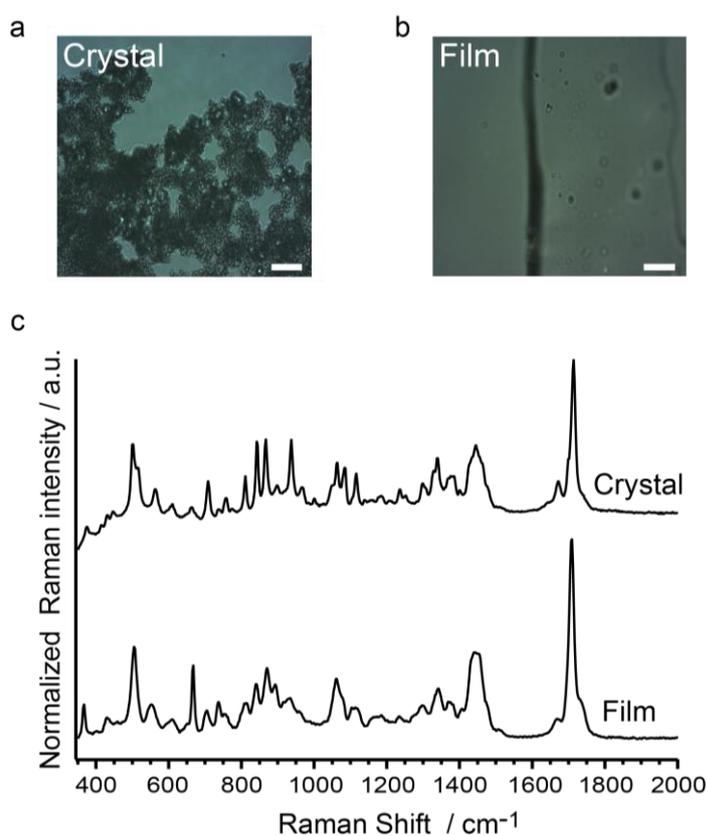


Figure. S3. Dependence of the Raman fingerprint on the morphology. (a) Transmission image of the polymer with crystal-like morphology (b) and film-like morphology. (c) The Raman spectra recorded for the film-like structure show slightly different fingerprint characteristics than the crystal-like structure. Scale bar: 10 μm .

Orientation dependence

Interestingly, the cycloaddition yield showed a strong dependence on the crystal orientation with respect to the light polarization of the laser used to induce the reaction. It is important to notice that the femtosecond laser employed in the two-photon process can only produce linear polarized light. A better understanding of the relationship between the crystal orientation and light polarization is therefore crucial. To investigate such orientation dependence, horizontal and vertical oriented monomer crystals were irradiated following the typical experimental conditions reported previously. Raman spectra were collected before and after irradiation from both samples and two characteristic spectral regions are shown in Figure. S4. Here, differences between irradiation performed on perpendicular and parallel oriented crystals are clearly visible. Specifically, after irradiation with perpendicular polarized light (Figure. S4a), bands corresponding to the cyclobutane ring clearly appeared, while peaks corresponding to the bis-thymine monomer completely disappeared. On the contrary, no spectral change could be detected after irradiation with parallel polarized light (Figure. S4b), suggesting that cyclization did not occur. Such dependence on the light polarization is likely caused by the anisotropic nature of the monomer crystals, as the bis-thymine molecules stack into one direction, crystallizing in rows. In this respect, the light polarization, combined with the crystal orientation, provide additional tools for a more precise control over the reaction.

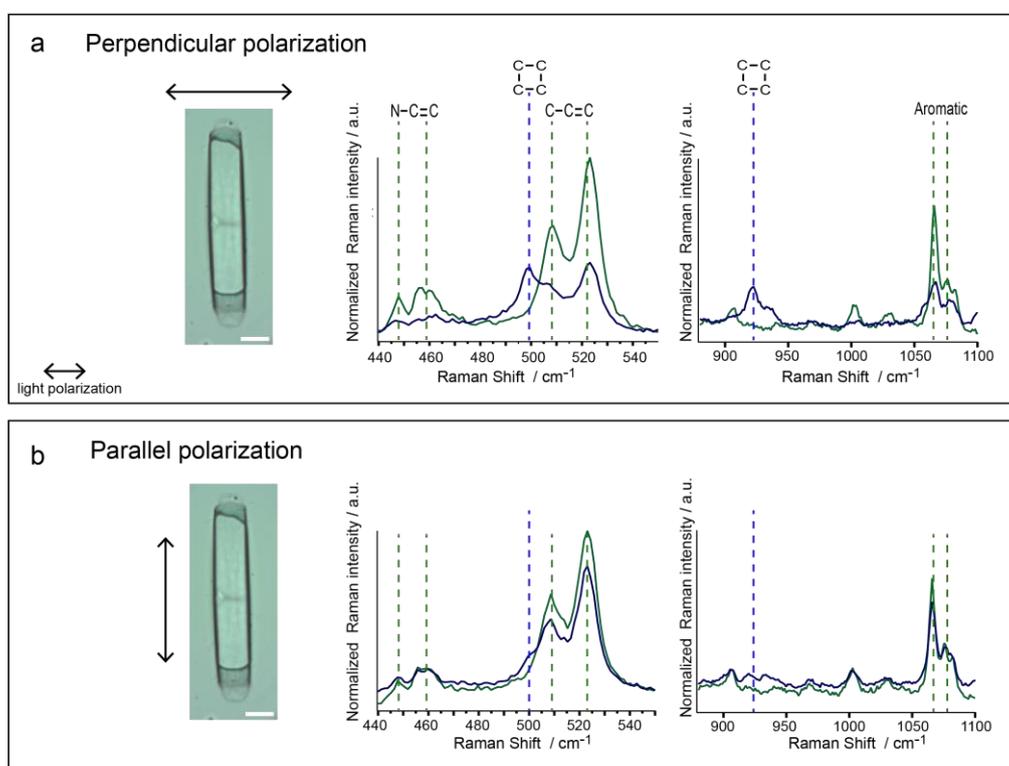


Figure. S4. Characteristic regions of the Raman spectra of two different orientated monomers. Results obtained for perpendicular and parallel polarization are displayed in Figure S4a and Figure S4b, respectively. The Raman spectra were obtained before (green lines, monomer) and after (blue lines, cyclic) femtosecond laser irradiation. Peaks corresponding to the characteristic vibrations of the cyclic and monomer structures are indicated with dotted lines (green: monomer, blue: cyclic). Scale bar: 5 μm .

Uv-Vis spectra

The UV-Vis absorption spectra of both the monomer and polymer samples, obtained via one-photon reaction, have been previously recorded by Prof. Saito et al. The spectra reveal no absorption from neither the monomer nor the polymer in the spectral region above 300 nm. These results further confirm the two-photon nature of the cycloaddition reaction.

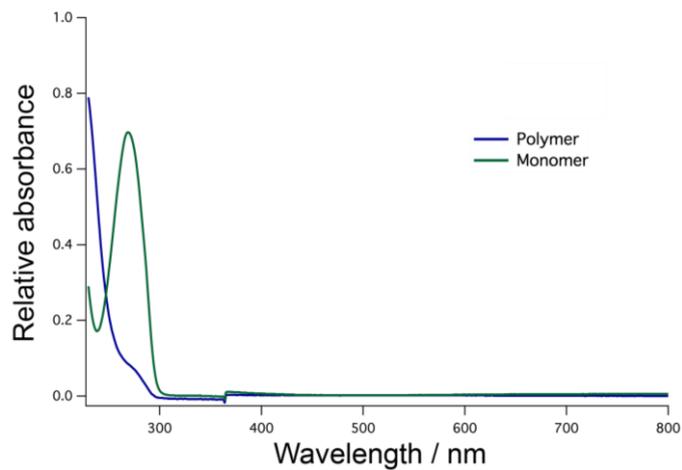


Figure. S5. UV-Vis absorption spectra recorded for the polymer (blue) and monomer (green) samples.

Reaction with 500 nm light

Monomerization using 500 nm light was also investigated. A monomer region was irradiated firstly with 600 nm irradiation and afterwards with 500 nm light. Interestingly, the Raman spectra recorded after irradiation with 500 nm showed a decrease of the cyclobutane related bands (498 cm^{-1} , 707 cm^{-1} and 922 cm^{-1}). However, no increase in the thymine related bands was observed (447 cm^{-1} , 463 cm^{-1} , 510 cm^{-1} , 524 cm^{-1} , 1638 cm^{-1} and 1656 cm^{-1}). These results suggest that the formation of the original monomer did not occur, as the characteristic bands of the thymine moieties did not reappear. Rather, the use of 500 nm light more likely resulted in a mere cleavage of the cyclobutane ring. This partial monomerization suggests that a new stable compound was formed upon irradiation with 500 nm light. Interestingly, subsequent irradiation of this new structure with 450 nm light produced the starting monomer, as confirmed by the Raman spectra collected after irradiation (Figure. S6).

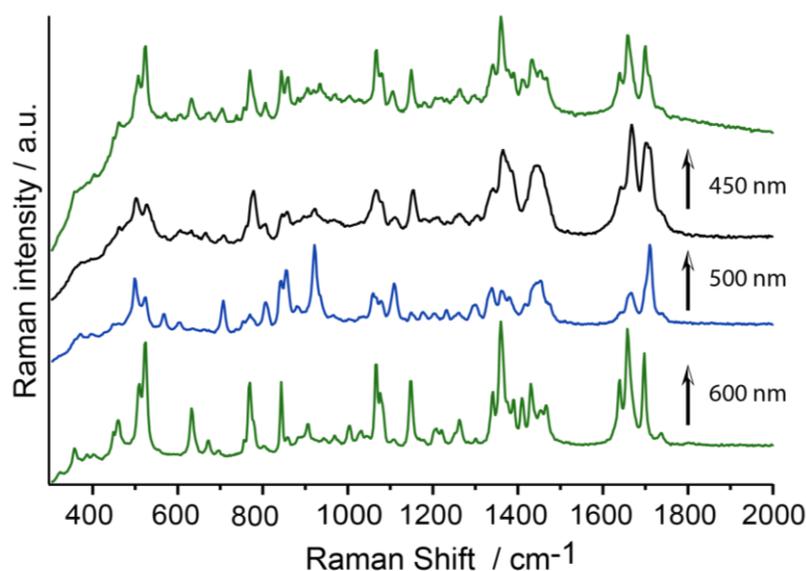


Figure. S6. Synthesis of the new chemical structure. Raman spectra have been recorded after each irradiation step. The starting monomer (green, bottom) was firstly irradiated with 600 nm light to yield the cyclic structure (blue). The same spot was subsequently irradiated with 500 nm light, obtaining the new structure (black). This new structure was hereafter fully converted into the starting monomer upon irradiation with 450 nm light (green, top).

References

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