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# Two-Photon-Absorption Triggered Release of 5-Fluorouracil from Isomer-Pure Polymer-Bound *Syn*-Head-to-Head Dimers for Novel Intraocular Lenses

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## Abstract

Different stereoisomers ofthe cytotoxic 5-fluorouracil (5FU)homodimers were synthesizedby photochemical [2+2]-cycloaddition and polymerized into MMA/HEMA to form a novel drug-loaded copolymer for intraocular lenses as irradiation-activated treatment for secondary cataract. Three isomers were obtained, and showed significant differences in cleavage efficiency on photo-cleavage via single-photon-absorption (SPA)and two-photon-absorption (TPA). The most efficient TPA cleavage rate was observed for the *syn*-head-to-head 5FU dimer, which was, consequently, used for drug loading of the polymeric material to obtain a drug-loaded material of higher efficiency compared to previous works. The light and thermal stability of the polymer were confirmed and multi-dose release of the drug in aqueous solution for possible repeated treatment of cataract was proven.

**Keywords:** Photo-triggered drug release, 5-fluorouracil, isomers, two-photon-absorption, [2+2]-cycloreversion, acrylic polymer.

## Introduction

Cataract is a disease caused by a dysfunction of eye metabolism,leading to an opacification of the natural lens.[1,2] About 20 million people worldwide have lost their vision due to cataract.[3,4]The standard treatment is the extracapsular cataract extraction (ECCE), *i.e.* the implantation of a posterior-chamber intraocular lens (IOL).[5,6] The most frequent complication associated with this treatment is the posterior capsule opacification (PCO) occurring in 15% to 50% of cases within three to five years after lens replacement.[7,8,9,10] The common treatment of PCO is laser capsulotomy,[11]which leads to thermal destruction of the posterior capsular bag.[12]Typical complications are retinal detachment and macular edema.[13]Several experiments using drugs such as colchicine, methotrexate, retinoic acid and 5-fluorouracil (5FU) have been made in animal models for PCO treatment with 5FU emerging as one of the most effective cytotoxic drugs.[14,15,16,17,18,19,20]

The pathogenesis of PCO is attributed to the persistence and response of lens epithelial cells (LECs). A fibrotic type of PCO appearsaccompanied by a capsular bag contraction. This depends on the proliferation and fibrous metaplasia of interior and equatorial LECs.[21,22]

In this work, we present a photochemically controlled drug release system for 5FU from 5FU homodimers formed by a Woodward-Hoffman [2+2]-cycloaddition reaction linking two molecules of the 5FU cytotoxic drug.To facilitate the reaction, a heptanoyl residue is temporarily attached to the 5FU molecule. Three out of four theoretically possible isomers were obtained in the photosynthesis (Figure. 1). The single-side deprotected homodimer with the highest two-photon-absorption photocleavage rate, i.e. the *syn*-hh-HD, is attached to the polymer backbone.

The formed polymer fulfills all the material demands for the manufacture of hydrophilic IOLs. During polymer hydration, the remaining heptanoyl group is hydrolyzed by adding phosphoric acid. The polymer is ready for use after solution is exchanged and brought to neutrality.

The 5FU dimer may be cleaved by light in a [2+2]-cycloreversion reactionwith wavelengths below 300 nm via single-photon-absorption (SPA) or with light in the visible range, e.g. 532 nm, via two-photon-absorption (TPA), the key to drug release from an implanted IOL. The released drug diffuses out of the IOL into the capsular bag to cause apoptosis of the LECs and treat PCO.

Compared to prior work from, e.g. Härtner et al. who used heptanoyl-modified 5FU dimers matrix-embedded into a hydrophobic polymer, the 5FU dimers from the new polymer do not show any leakage from the polymer without photo excitation. In addition, only 5FU, and no auxiliary compound, is released because the heptanoyl residue is cleaved off the hydrophilic polymer during swelling. Finally, the new polymer may be steam autoclaved, which is the most simple and common sterilization method. In other work[23,25] coumarin was used as a linker molecule to attach the 5FU to the polymer backbone. The problem with coumarin is that the lactone ring may be opened by pH changes or thermal treatment, which changes the photochemical cleavage sensitivity and cleavage pattern.[26]

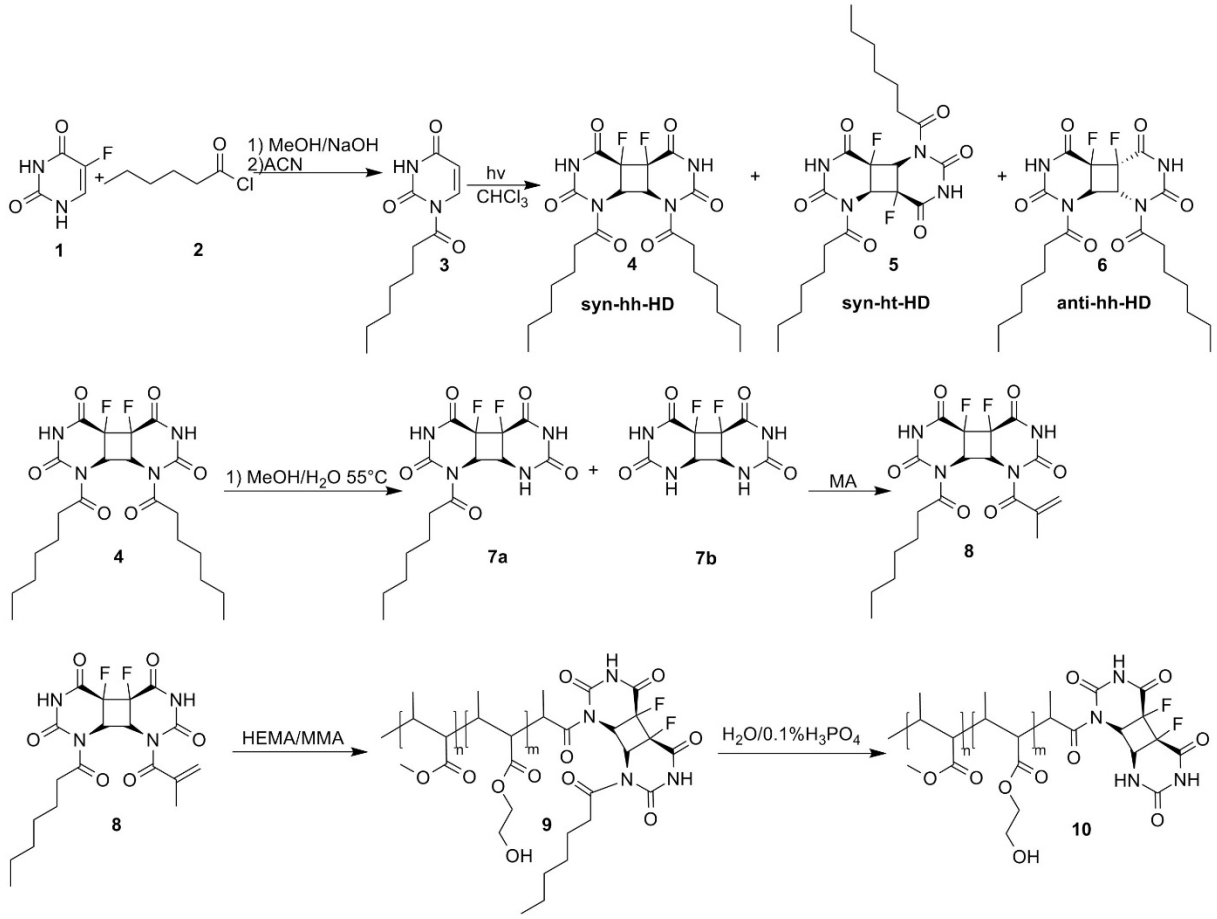


**Figure 1.** Synthesis of the drug release polymer. In the dimerization of (3) three out of four possible stereoisomers are obtained, *syn*-head-to-head-HD (4), *syn*-head-to-tail-HD (5), and *anti*-head-to-head-HD (6). After single side deprotection of (4) methyl-methacrylate (MMA) is attached to (7a) which is copolymerized with MMA/HEMA to form (9). After acid catalyzed hydrolysis of the remained heptanoyl group the final polymer (10) is obtained.

The 'dual-use' of 5FU as linker as well as drug to be released solves both problems and brings these materials closer to application.

## Materials and Methods

### Materials

All materials were purchased from Acros Scientific,Air Liquide, Fluorochem (TBDMS-Cl), Melrob, Sigma Aldrich (heptanoylchloride, EDC, DMAP)and were used as received.

### Photochemical syntheses

For the photochemical synthesis of homodimers an UV-reactor was used which was equipped with 12 fluorescent tubes(40W-R/35, Bodytone, Isolde). All reactions were carried out under Schlenk-conditions with argon as protective gas.

### Analytics

The reactions were characterized by NMR recorded on a Bruker DRX-500 FT-NMR (500 MHz, $^{1}$H-NMR, NOESY, COSY, HMBC) andon a Bruker,Advance 300 spectrometer (75 MHz $^{13}$C-NMR). Analytical RP-HPLC measurements were done on an Ultimate 3000 system from Dionex, equipped with a Nucleosil column (Prontosil RP-C18, 5.0 µm, 250 4mm, Bischoff Chromatography), a quaternary low pressure gradient pump, a column oven, and a diode array detector(DAD). The eluent for isocratic separation was a 75:25 mixture of acetonitrile:water (ultra-pure water 0.065µScm$^{-1}$ from TKA ultra-pure water purification system) which was acidified with 300µl L$^{-1}$concentrated $H_3PO_4$.

The isomeric products were purified by a preparative RP-HPLC system consisting of a P680 HPLC pump, an AD 25 absorbance detector (bothfrom Dionex), and a YMC-Pack ODS-A column, 250 mm 30mm, 5µm.Isocratic purifications were done with 70:30 acetonitrile:water with 10µl $H_3PO_4$ conc. added per liter.
LC/MS analysis was done on an ESI/MS LCQ DUO mass spectrometer (Thermoelectron) with a Nucleosil 100-3-C18 column (3µm, 250 4mm) from Bischoff Chromatography, Germany.
UV/Visspectra were recorded on a Lambda 35 spectrometer from Perkin Elmer in the range of 190 nm to 800 nm in steps of 1 nm. Measurements were done in quartz cuvettes (QS, Hellma; UQ, Portman Instruments) with 10 mm path length.
Sterilization of the polymers was done in an autoclave at 121 C and 2 bar (2540 EL, Tuttnauer).

## Single-photon-absorption (SPA) induced cycloreversion

The photochemical cycloreversion of homodimers in solution and polymers was achieved by irradiation with wavelengths of 266 nm in acetonitrile. 2.0 ml of a 0.21 mmol $l^{-1}$ solution of isolated isomer were irradiated with an $UV_{266}$ LED (UV-LUX260) having an energy of $5.1\times10^{-4}$ W/$cm^2$ (number of photons per second: 6.80 $10^{14}$) at room temperature.The intensity of the diode as well as the light transmitted through the sample was measured by a photodiode from Hamamatsu (Modell: S133BQ1010) combined with a Keithley 485 Autoranging Picometer.

## Two-photon-absorption (TPA) induced cycloreversion

TPA measurements were made with 2.7 ml of 2.1mmol $l^{-1}$ stirred solutions in acetonitrile of individual homodimersand of polymers at room temperature under irradiation with a 532 nm pulsed Nd:YAG laser (Infinity 40-100, Coherent) with a pulse length of 3 ns. The laser beam had a diameter of 5.5 mm with a flat-top profile and a repetition rate of 40 Hz. The average energy was measured using a Fieldmaster GS power meter equipped with a 80V detector head (both Coherent).The photo-cleavage progress was traced photometrically by measuring the absorbance change at 266 nm.

## Synthetic procedures

## Synthesis of 1-heptanoyl-5-fluorouracil (H5FU)

5FU ishardly soluble in most organic solvents. A heptanoyl group was attached as protection groupand to improve solubility. The unprotected amino group of 5FU interferes with the photochemical reaction and reduces the yield. The reaction was done as described earlier.[28]
$^1$H-NMR (300 MHz, DMSO-d6): /ppm= 7.25 (s, 1H), 7.07 (s, 1H), 2.60-2.56 (m, 2H), 2.69-1.61 (m, 2H), 1.42-1.29 (m, 6H), 1.01-0.97 (t, J= 6.6 Hz, 3H). $^{13}$C NMR result (75MHz, DMSO-d6): /ppm= 177.04, 157.39, 150.43, 138.70, 115.82, 37.53, 31.65, 29.74, 22.50, 22.94, 14.02. LC/MS (ESI): m/z calc. 242.12 g/mol, found 242.23 g/mol ($M^+$).

## Preparation of 1-heptanoyl-5-fluorouracil-homodimers (H5FU-HD)

The dimerization of H5FU (3)was done in a Rayonett type photoreactor with wavelengths above 300 nm. In each test tube 1.0 g H5FU (3,0.004128 mol) was dispersed in 15 ml chloroformwith3.0 mg photosensitizer benzophenone (0.0165 mmol) added.The white suspension was degassed for 10 min with argon and thenirradiated with UV light for 48-72 h. The reaction progress was monitored by HPLC. The reaction was stopped as soon as no further dimer formation was observed. The tubes were combined, evaporated and dried. The crude product wasdissolved in acetonitrile, filtered and purified viaisocratic preparative HPLC. The homodimerswere received as white solids(H5FU-HD isomer 4: 0.34 g, 0.70 mmol, 34 %; isomer 5:0.28 g, 0.58 mmol, 28 %; isomer6:0.18 g, 0.32 mmol, 17.5%). Stereochemistry was determined from 2D NMR: COSY, NOESY, and HMBC (for details see supporting information).
NMR data of syn-hh-HD isomer (4):1H NMR (300 MHz, DMSO-d6): /ppm= 11.69 (s, 2H), 5.39 – 5.33 (m, 2H), 3.01 – 2.94 (m, 2H), 2.85 (ddd, J = 17.1, 8.2, 6.5 Hz, 2H), 1.61 – 1.54 (m, 4H), 1.29 (dd, J = 5.9, 4.1 Hz, 12H), 0.88 (s, J = 6.7 Hz, 6H).13C NMR: (75MHz, DMSO-d6): /ppm= 174.05, 162.84, 149.54, 57.36, 37.73 31.02 28.03 24.13 21.96 13.87. 19F NMR (282MHz, DMSO-d6): /ppm= -171.39, -151.38.
LC/MS (ESI): m/z 484.21 g/mol (calc.), found 484.2 g/mol ($M^+$).
NMR dataof*syn*-ht-HD isomer (5):$^1$H NMR (300 MHz, DMSO-d6): /ppm= 11.81 (s, 2H), 5.99 – 5.85 (m, 2H), 2.96 – 2.88 (m, 2H), 2.82 (m, 2H), 1.66 – 1.50 (m, 4H), 1.37 – 1.22 (m, 12H), 0.86 (d, *J* = 7.0 Hz, 6H).$^{13}$C NMR(75MHz, DMSO-d6): /ppm= 173.95, 161.94, 148.50, 56.06, 37.82, 31.02, 28.09, 24.06, 21.96, 13.88.$^{19}$F NMR (282MHz, DMSO-d6): /ppm= -204.81, -188.41.
LC/MS (ESI): m/z 484.21 g/mol (calc.), found484.21 g/mol ($M^+$).
NMR data of *anti*-hh-HD isomer (6):$^1$H NMR:(300 MHz, DMSO-d6): /ppm= 11.86 (s, 2H), 5.55 – 5.49 (m, 2H), 2.75 (t, *J* = 7.3 Hz, 4H), 1.50 (dd, *J* = 13.9, 6.7 Hz, 4H), 1.32 – 1.23 (m, 12H), 0.88 (dd, *J* = 7.0, 4.7 Hz, 6H).$^{13}$C NMR (75MHz, DMSO-d6): /ppm= 173.75, 161.98 150.10 53.77 53.53 38.13 28.57 24.51 22.43 14.32.$^{19}$F NMR (282MHz, DMSO-d6): /ppm= -151.95, -157.86.
LC/MS (ESI): m/z 484.21 g/mol (calc.), found 484.1 g/mol ($M^+$).

## Deprotection of H5FU homodimers

The heptanoyl protection group of the homodimer (4)(154.9 mg, 0.32 mol) was partially removed under mild conditions by stirring the product in a methanol/water mixture (60/40;200ml) for 96 h at 55 C. The progress of the reaction was monitored by HPLC. The reaction was stoppedby cooling. The double-deprotected product (7b) precipitates and was removed by filtration.The crude product was purified via isocratic preparative HPLC. The eluent was evaporated, the material dissolved in methanol and crystallized. The single-sidedeprotected homodimer(7a) as main product was obtained as a white solid (68 mg, 0.14 mmol, 44 %).

LC/MS (ESI): m/z calc. single deprotected: 372.12g/mol ($M^+$), found 371.1 g/mol; double deprotected:260.0 g/mol ($M^+$), found 263.1 g/mol.

### Attachment of methyl-methacrylate to single-side deprotected syn-hh-H5FU-HD

For covalent immobilization of the dimer, methyl-methacrylate was covalently bound to the deprotected amino group of *syn*-hh-HD (7).60 mg homodimer (0.12 mmol), 40.38 mg ethyl-(N',N'-dimethylamino)propylcarbodiimide hydrochloride (EDC, 1.7 eq, 0.21 mmol) and 3.03 mg 4-(N,N-dimethylamino)pyridine (DMAP, 0.2 eq., 0.025 mmol) were dissolved in dry dichloromethane (50ml) at 0 C under argon atmosphere. 11.50µl methacrylic acid (1.1 eq., 0.14mmol) were slowly added under Schlenk conditions and stirred for 24-48 h.The progress was monitoredvia HPLC. The crude product was extracted with 5% $NaHCO_3$ and brine. The organic phase was evaporated, dried in vacuo. Ayield of88 mg (84%) of a slightly brown solid was received. The product was analyzed via NMR and LC/MS.

NMR data of *syn*-hh-HD-MMA(8):1H NMR (300 MHz, DMSO-d6): /ppm= 8.39 (s, 1H), 8.07 (s, 1 H), 6.77 (m, 1H), 5.24-5.21 (m, 1H), 4.99-4.97 (t, $J$= 0.40 Hz, 2H), 4.84 (m, 2H), 2.85 (s, 1 H), 1.88 (t, $J$ = 7.0, 4.7 Hz, 3H).$^{13}$C NMR (75MHz, DMSO-d6): /ppm=174.08, 164.21, 163.97, 163.07, 162.76, 151.32, 151.13, 149.94, 124.94, 85.50, 81.89, 57.92, 56.35, 56.03, 31.07, 28.06, 24.22, 21.99, 13.92. $^{19}$F NMR result (282MHz, DMSO-d6): /ppm= -179.3.

LC/MS calculated 328.06 g/mol ($M^+$), found 329.5 g/mol.

### Photochemical co-polymerization of syn-hh-5FU-HD-MMAwith HEMA/MMA

65 mg of *syn*-hh-5FU-HD-MMA(0.2 mmol) (8)were dissolved in 2.6 g of a standard 20:80 MMA:HEMA mixture with ethylene-glycol-dimethacrylate (EGDMA) as crosslinker and campherquinone and ethyl-4-dimethylaminobenzoate as photostarters.[24,27] The polymer mixture was degassed and filled into the polymerization form (3.6 3.6 0.25 cm). The polymerization was started by irradiation with 470 nm light. An optically transparent plate, containing 2.5 w/w% of drug loaded monomer was obtained. A second series of samples was preparedcontaining 1 % of the UV-absorber 2(4-benzoyl-3-hydroxy-phenoxy)ethyl-acrylate (BHP-EA) in addition.

### Polymer sample preparation

The polymer was cut into round blanks of 8 mm diameter and 0.25 mm thickness by a $CO_2$-Laser (Skylaser 9060-80, 80 W $CO_2$-Laser 4-10.6 µm, Pfeiffer).

### Hydration of polymer

The samples were incubated for 21 days at room temperature inwater for hydration and for ensuring that the photo starter completely diffused out of the polymer topreventany interferences of the absorption of the starter with the released drug during UV/Vis spectroscopy. To ensure that the heptanoyl group was detached the water was acidified with0.1 % $H_3PO_4$. UV/Vis spectra of the incubation solution were taken regularly. No drug diffused out of the polymerduring hydration.

## Results and Discussion

The photochemical synthesis of dimers produced three of four possible isomers. For the drug release, the dimers areto be cleaved photochemically. The different isomers show quite different photochemical efficacies for [2+2]-cycloreversion triggered by SPA as well as TPA. In cases where the mixture of isomers is used in the drug release polymer, first the more light sensitive dimers are cleaved and then the less sensitive ones. This means, the amount of drug released per light dose changes constantly. To circumvent this, we prepared the drug release polymer with one isolated isomer only, having the highest cleavage efficiency under TPA conditions.

### SPA-induced cycloreversion of the H5FU-HD isomers

The *syn*-hh-HD, *syn*-ht-HD and *anti*-hh-HD homodimers from H5FU were photosynthesized in a Rayonett reactor and purified by preparative HPLC (see Figure. 1). The *anti*-ht-HD was not observed. Their stereochemistry was determined by NMR spectroscopy.[28] All the H5FU dimers are less soluble in polar solvents like water than the monomers. Irradiation of the homodimers with 266 nm in acetonitrile causes the cycloreversion of the homodimers (Figure. 2).

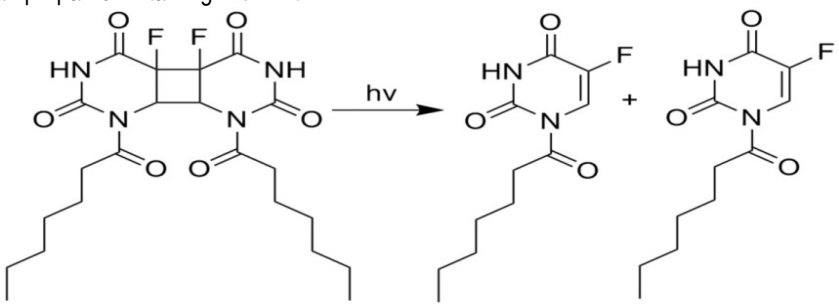


**Figure 2.**Photocleavage of H5FU homodimers. The photocleavage (hv) may be accomplished either by single-photon-

absorption in deep UV or by two-photon-absorption with wavelengths in the visible.

As the HPLC analysis tracing the SPA-induced cycloreversion of the homodimers uses acidified water in the eluent, both H5FU and 5FU are observed because the heptanoyl group is easily hydrolyzed.The difference spectra in Figure 3a show the time-dependent and energy-dose-dependent increase in the absorbance at 260 nm, the maximal absorbance of H5FU.The dimers merely have any absorbance at 260 nm, so the absorbance is characteristic of the H5FU (see supporting information S1).

The photocleavageefficiency is characterized by the single photon quantum yield $_{SPA}$which is defined as the ratio of the number of dimer molecules $n_{mol}$cleaved to the number of photons$n_{phot}$ absorbed[26] with $E_{abs}$ the absorbedenergy, $\lambda$ the wavelength, $c_{hv}$ the speed of light, $h$ Planck's constant, $\Delta c/\Delta t$the slope of the time-dependent concentration change of H5FU, $V$the volume of the test solution, and $N_A$ the Avogadro constant.

$$\Phi_{SPA} = \frac{n_{mol}}{n_{phot}}; \quad \frac{n_{phot}}{E_{abs}} = \frac{\lambda}{c_{hv} \cdot h}; \quad n_{mol} = \frac{\Delta c}{\Delta t} \cdot V \cdot N_A$$

The number of cleaved dimer molecules was calculated from the initial reaction rate $v_0$ which was obtained from the linear fit of the concentration as a function of time (Figure.3b).

The concentration changes, the initial reaction rate $v_0$and the quantum yield of the isomer cleavage $_{SPA}$ are summarized in Table 1. H5FU *syn*-hh-HD $_{SPA}$ shows the highest quantum yield, followed by H5FU *syn*-ht-HD and H5FU *anti*-hh-HD. It should be mentioned that the absorption of the dimers at 266 nm is quite low (for reference see supporting information Figure S1) but we have chosen this wavelength because the two-photon-absorption induced cycloreversion is done at 532 nm.

As already known from other dimers,[29,30] the configuration influences activation barrier for cycloreversion. Both hh dimers show significantly better SPA quantum yields than the ht isomer.

*TPA-induced cycloreversion of the H5FU-HD isomers*

Solutions of the homodimers(4), (5), and (6)in acetonitrile (c=0.21 mmol/L) were irradiated with intense 532 nm pulses (40 Hz, 3 ns) which cause TPA-induced cleavage of the homodimer (Figure.4a). The cleavage is monitored at 266 nm. An increase of the absorption at this wavelength proves the cleavage of the cyclobutane ring of the homodimer. The TPA cross section was measured at fourdifferent energies (90 mJ, 100 mJ, 110 mJ, 120 mJ) (Figure.4b). The rising concentration of released H5FU was plotted against the irradiation time for different pulse energies to obtain the initial reaction rate $v_0$. In Figure 4c the double logarithmic plot of the initial reaction rate $v_0$as a function of the pulse energies P is presented. A linear slope of 2.23 results, which confirms generally the two-photon nature of the cycloreversion.

The results for all three isomers are summarized in Table 2. Again the hh isomers are more efficient than the ht isomers, but compared to SPA the difference between *syn*-hh and *anti*-hh is more significant.

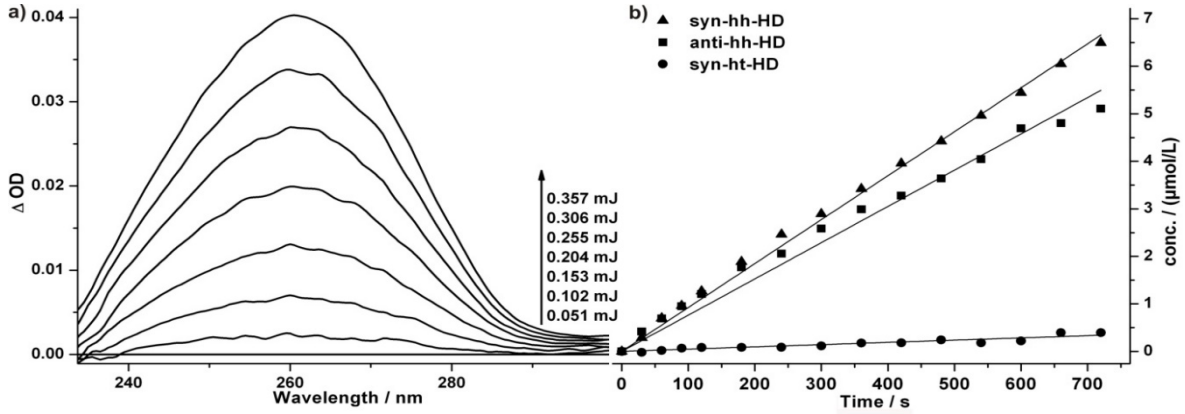


**Figure 3.** (a) Difference spectra of *syn*-hh-HD in solution taken after different doses of irradiation. An increase in absorbance change ind free 5FU. (b) SPA quantum yield ( $_{SPA}$) determination for all three isolated stereoisomers.

**Table 1.**Molar extinction coefficients and photochemical cleavage parameters of H5FU homodimers via SPA.

| Stereoisomer | $_{266}$ ($L \cdot mol^{-1} \cdot cm^{-1}$) | $v_0 = \Delta c/\Delta t$ ($mol \cdot sec^{-1} \cdot L^{-1}$) | $n_{mol}$ ($s^{-1}$) | $_{SPA}$ (%) |
|---|---|---|---|---|
| H5FU | 6,279.07 ± 43.04 | | | |
| *syn*-hh-HD | 346.58 ± 31.05 | 8.92 $10^{-9}$ | 8.42 $10^{12}$ | 2.76 |
| *syn*-ht-HD | 163.97 ± 4.45 | 4.96 $10^{-10}$ | 0.99 $10^{12}$ | 0.98 |
| *anti*-hh-HD | 178.42 ± 11.62 | 6.99 $10^{-9}$ | 7.06 $10^{12}$ | 1.74 |

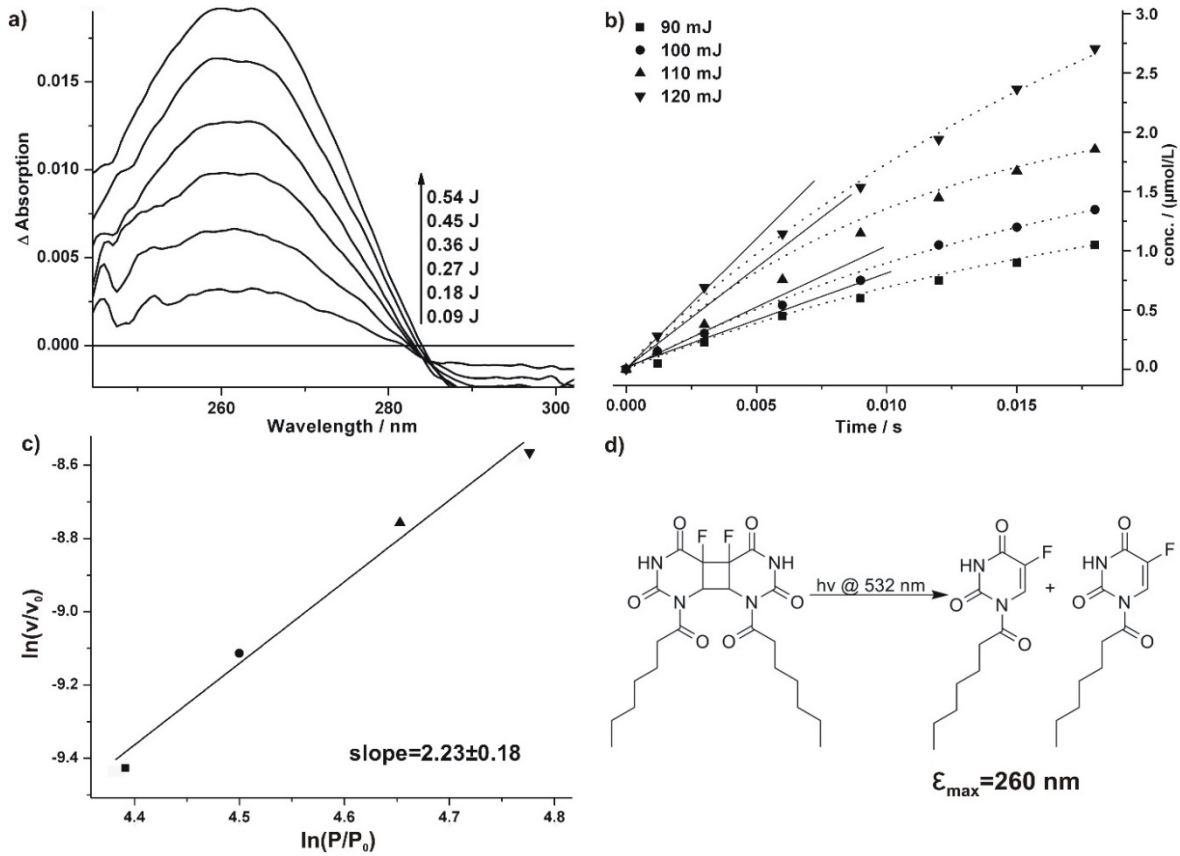


Figure 4. Two-photon-absorption induced cleavage of *syn*-hh-HD (4) in solution. (a) UV/Vis difference spectra accompanying the irradiation with Nd:YAG laser as a function of irradiation energy. (b) Released H5FU at different pulse intensities (40Hz and 3 ns pulse length). (c) Double logarithmic plot of the applied laser intensities P versus the initial rate $v_0$ (slope 2.23). (d) Scheme of the photocleavage reaction of homodimer.

Table 2.Photochemical cleavage parameters of H5FU homodimers via TPA

| Stereoisomer | pulse energy | $v_0$ | | average |
|---|---|---|---|---|
| | (mJ) | mol/L | GM | GM |
| *syn*-hh | 90 | $9.09\ 10^{-5}$ | 0.52 | 0.58 |
| | 110 | $1.34\ 10^{-4}$ | 0.59 | |
| | 118 | $1.58\ 10^{-4}$ | 0.57 | |
| | 130 | $2.00\ 10^{-4}$ | 0.63 | |
| *syn*-ht | 98.5 | $4.94\ 10^{-5}$ | 0.18 | 0.17 |
| | 110 | $6.19\ 10^{-5}$ | 0.18 | |
| | 118 | $6.49\ 10^{-5}$ | 0.18 | |
| | 131 | $7.73\ 10^{-5}$ | 0.16 | |
| *anti*-hh | 92.5 | $7.98\ 10^{-5}$ | 0.23 | 0.25 |
| | 110 | $1.10\ 10^{-4}$ | 0.26 | |
| | 120 | $1.54\ 10^{-4}$ | 0.27 | |
| | 130 | $1.88\ 10^{-4}$ | 0.26 | |

## SPA-and TPA-induced drug release from polymer

Relevant for the application is the drug release from the hydrated polymer. Both SPA and TPA induce the release of 5FU (Figure. 5).

Because there is an UV absorber in the polymer, the SPA induced cleavage is much less efficient, because most of the light is absorbed by the UV absorber and not by the dimers.

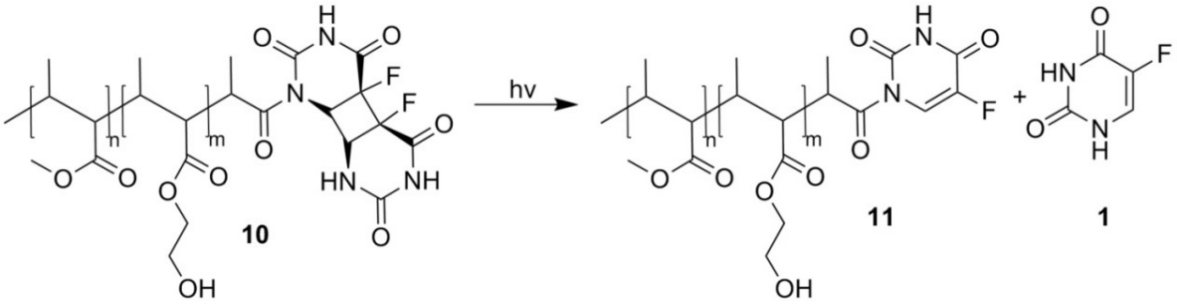


**Figure 5.** SPA and TPA cleavage of the 5FU homodimers leaves one 5FU at the polymer and one 5FU molecule released

For SPA-induced drug release polymer blanks were irradiated with an UV LED for 2 min (Figure. 6a)in physiological salt solution (0.9 % NaCl).The drug diffusion from the polymer into the solution was monitored via UV/Vis until equilibrium was reached. This procedure was then repeated. The drug release rate from the polymer was determined to be 1.1 $10^{-4}$µg $s^{-1}$ and the total drug release after 60 min was 0.48µg.

For analysis of TPA-induced drug release ahydrated polymer blank (1 0.6 0.25 cm) was mounted in a home-made holder inside a quartz cuvette and irradiated with 532 nm pulses. UV/Vis spectra were taken to monitor the 5FU release out of the polymer. The irradiation of the polymer was repeated three times. In total 4.38 µg 5FU were released (Figure. 6b). HPLC analysis (Figure. S7) shows that only 5FU is released.

The released doses are more than adequate for cataract treatment to cause the inhibition and cell death of LECs.[17,31] This experiment demonstrates that a multidose drug release is possible meaning that repeated secondary cataract treatment is possible.

The cleavage of the three different homodimers of the cytotoxic drug analogue H5FU yielded a stereoisomer-dependent cycloreversion efficiency of SPA and TPA activation. Both head-to-headconfigurations show a significantly greater cleavage efficiency compared to the head-to-tail configuration due to sterical effects of activation by the photons, and the highest efficiency was found for the *syn*-head-to-head dimer. The most efficient isomer should be used as drug carrier, as it is easy to obtain a drug-loaded polymer for secondary cataract treatment that may be activated with the lowest possible energy dose in the eye. The light fastness and the stability against autoclaving, the possible multi-dose release of drug from the polymer loaded with the high-efficiency isomer, and the release of a sufficient 5FU concentrations in aqueous solutions for removing the epithelial cells in the capsular bag during secondary cataractwas experimentally proven.[32]The system is now ready for moving to cell tests and in-vivo experiments.

## Conclusions

## References


[1]. Lin MT, Flint Beal M.Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases.Nature2006;443:787-795.

[2]. Truscott RJW. Human cataract: The mechanism responsible; light and butterfly eyes.Int. J. Biochem. Cell Biol. 2003;35:1500-1504.

[3]. WHO, Vision 2020 the Right to Sight, Action Plan 2006-2011. http://www.who.int/mediacentre/factsheets/fs282/en/; http://www.lighthouse.org/research/statistics-on-vision-impairment/prevalence-of-vision-impairment/

[4]. Apple DJ, Auffarth GU, Peng Q, Visessook N.Foldable Intraocular Lenses, Evolution, Clinicopathologic Correlations, Complications. Thorofare, NJ, U.S.A.: Slack Inc.; 2000.p. 157-215.

[5]. Apple DJ, Ram J, Foster A, Peng Q.Elimination of cataract blindness: a global perspective entering the new millennium. Surv. Ophthalmol. 2000;45:1-196.

[6]. Apple DJ, Solomon KD, Tetz MR, Aissa EI, Holland EY, Legler UF, Tsai JC, Castaneda VE, Hoggatt JP, Kostick AM.Posterior capsule opacification.Surv. Ophthalmol. 1992;37:73-116.

[7]. Werner L, Apple DJ, Pandey SK. Postoperative proliferation of anterior and equatorial lens epithelial cells. In: Buratto L, Osher RH, Masket S., editors. Cataract surgery in complicated cases. Thorofare, NJ, U.S.A.: Slack Inc.; 2000.p. 399-417.

[8]. Schaumberg DA, Dana MR, Christen WG, Glynn RJ.A systematic overview of the incidence of posterior capsule opacification. Ophthalmology 1998;105:1213-1221.

[9]. Apple DJ, Peng Q, Visessook N, Werner L, Pandey SK, Escobar-Gomez M, Ram J, Auffarth GU.Eradication of posterior capsule opacification: documentation of a marked decrease in Nd:YAG laser posterior capsulotomy rates noted in an analysis of 5416 pseudophakic human eyes obtained postmortem. Ophthalmology 2001;108:505-518.

[10]. StagerJr.DR, Wang X, WeakleyJr.DR, Felius J.The effectiveness of Nd:YAG laser capsulotomy for the treatment of posterior capsule opacification in children with acrylic intraocular lenses. JAAPOS2006;10:159-163.

[11]. Fankhauser F, Lörtscher H, van der Zypen E.Clinical studies on high and low power laser radiation upon some structures of the anterior and posterior segments of the eye: Experiences in the treatment of some pathological conditions of the anterior and posterior segments of the human eye by means of a Nd:YAG laser, driven at various power levels.Int.Ophthalmol. 1982;5:15-32.

[12]. Koch DD, Liu JF, Gill EP, Parke DW.Axial myopia increases the risk of retinal complications after neodymium-YAG laser posterior capsulotomy. Arch. Ophthalmol.1989;107:986-990.

[13]. Steinberg EP, Javitt JC, Sharkey PD, Zuckerman A, Legro MW, Anderson GF, Bass EB, O'Day D.The Content and Cost of Cataract Surgery. Arch. Ophthalmol.1993;111:1041-1049.

[14]. Tetz MR, Ries MW, Lucas C, Stricker H, Völcker HE.Inhibitionof posterior capsule opacification by an intraocular-lens-bound sustained drugdelivery system: an experimental animal study and literature review. J. Cataract Refract. Surg. 1996;22: 1070-1078.

[15]. Legler UFC, Apple DJ, Assia EI, Bluestein EC, Castaneda VE, Mowbray SL. Inhibition of posterior capsule opacification: The effect of colchicine in a sustained drug delivery system. J. Cataract Refract Surg. 1993;19:462-470.

[16]. Ruiz JM, Medrano M, Alió JL. Inhibition of Posterior Capsule Opacification by 5-Fluorouracil in Rabbits. Ophthalmic Res. 1990;22:201-208.

[17]. Ismail MM, Alió JL, Ruiz Moreno JM.Prevention of secondary cataract by antimitotic drugs: experimental study. Ophthalmic Res. 1996;28:64-69.

[18]. Power WJ, Neylan D, Collum LMT.Daunomycin as an inhibitor of human lens epithelial cell proliferation in culture. J. Catract Refract. Surg. 1994;20:287-290.

[19]. Brown JD, Pearson PA, Blandford D, et al. Controlled release 5-FU delivery systems: Release studies and HPLC assays [abstract].Invest. Ophthalmol. Vis. Sci. 1991,32 (Suppl):1293.

[20]. Werner L, Pandey SK, Escobar-Gomez M, Visessook N, Peng Q, Apple DJ.Anterior capsule opacification: a histopathological study comparing different IOL styles. Ophthalmology 2000;107:463-471.

[21]. Sacu S, Menapace R, Buehl W, Rainer G, Findl O.Effect of intraocular lens optic edge design and material on fibrotic capsule opacification and capsulorhexis contraction.J. Cataract Refract. Surg. 2004;30:1875-1882.

[22]. Härtner S, Kim HC, Hampp N.Phototriggered release of photolabile drugs via two-photon absorption-induced cleavage of polymer-bound dicoumarin. J. Polym. Sci.: Part A: Poly. Chem. 2007;45:2443-2452.

[23]. Kehrlößer D, Behrendt PJ, Hampp N. Two-photon absorption triggered drug delivery from a polymer for intraocular lenses in presence of an UV-absorber. J. Phys. Chem. A2012;248:8-14.

[24]. Träger J, Kim HC, Hampp N.Ophthalmology: Two-photon treatment. Nature Photonics 2007;1:509-511.

[25]. Härtner S, Kim HC, Hampp N. Dimeric drug depots forms for photo-induced drug release. Proc. SPIE 2004;5323:382-389.

[26]. Liese J, Hampp N.1,1-Dimethylnaphtalenon-dimers as photocleavable linkers with improved two-photon-absorption efficiency and hydrolitic stability. J. Photochem. Photobiol. A 2010;209:128-134.

[27]. Shim SC, Lee SH.Photosensitized Cyclodimerization of 5-Fluorouracil. Photochem. Photobiol. 1979;29:1035-1038.

[28]. Schaumann E, Ketcham R.[2 + 2]-Cycloreversions. Angew. Chem., Int. Ed. Engl. 1982;21:225-247.

[29]. Yonezawa N, Yoshida T,Hasegawa M. Symmetric and asymmetric photocleavage of the cyclobutanrings in head-to-head coumarin dimers and their lactone-opened derivatives. J. Chem. Soc. Perkin Trans. 1983;1:1083-1086.

[30]. Fernandez V, Fragoso MA, Billotte C, Lamar P, Orozco MA, Dubovy S, Willcox M, Parel JM.Efficacy of various drugs in the prevention of posterior capsule opacification: experimental study of rabbit eyes. J. Cataract. Refract. Surg.2004;30:2598-2605.

[31]. Su X, Li S, Zheng J. Inhibition of rabbit lens epithelial cells.Zhonghua Yan Ke Za Zhi.(Chinese journal of ophthalmology)1996;32:339-341.Acknowledgements

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## Supporting information

Preparative isolation of H5FU-HD isomers
2D-NMR of H5FU-HD isomers
Thermal stability of polymer - Steam pressure sterilization
Analysis of photo-triggered released compounds

The isomeric were separated and purified on a preparative RP-HPLC (P680 HPLC pump, AD25 absorbance detector, both Dionex) equipped with a YMC-Pack ODS-A column (250 mm 30 mm, 5 µm). Isocratic elution with 70:30 acetonitrile:water with 10µl $H_3PO_4$ conc. added per liter.

## Preparative isolation of H5FU-HD isomers

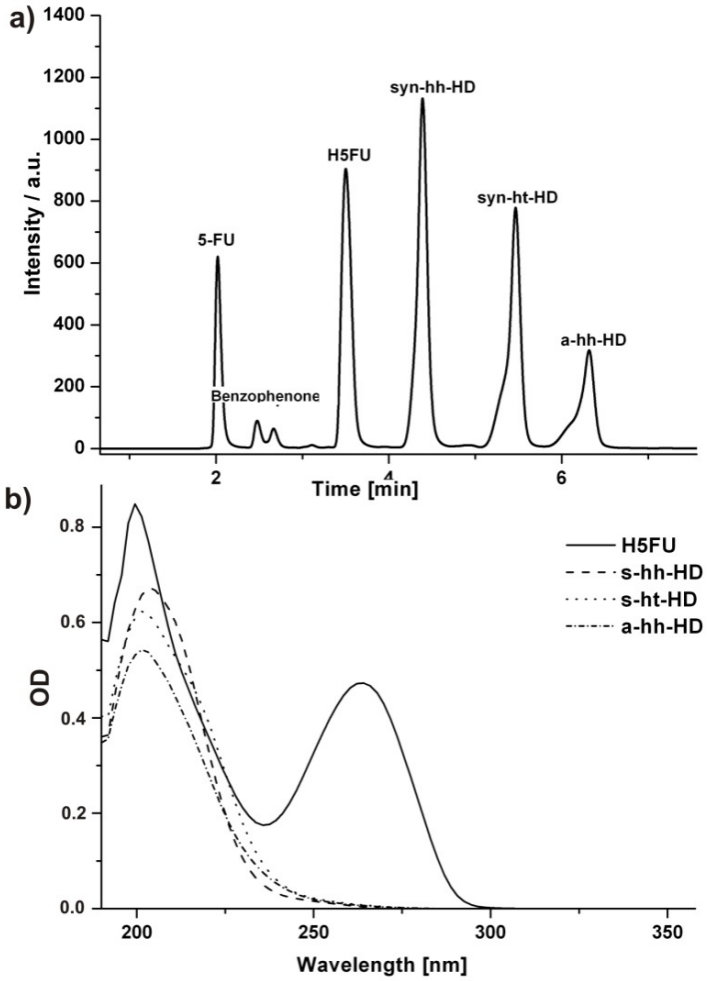
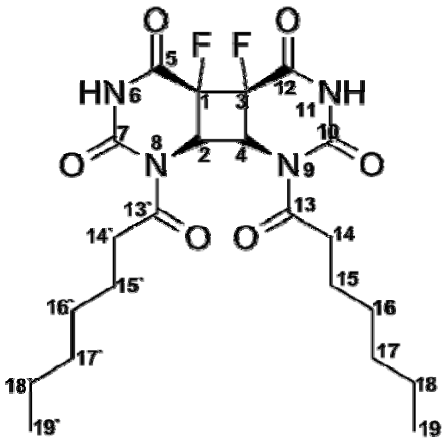



**Figure S1:** (a) Isolation of 5FU-isomers via preparative isocratic HPLC. Retention time of *syn*-hh-HD 4.34 min; *syn*-ht-HD 5.4 min; *anti*-hh-HD 6.23 min. (b) Absorption spectra of H5FU at 0.018 mmol/L and the homodimers at 0.021 mmol/L in acetonitrile at room temperature.

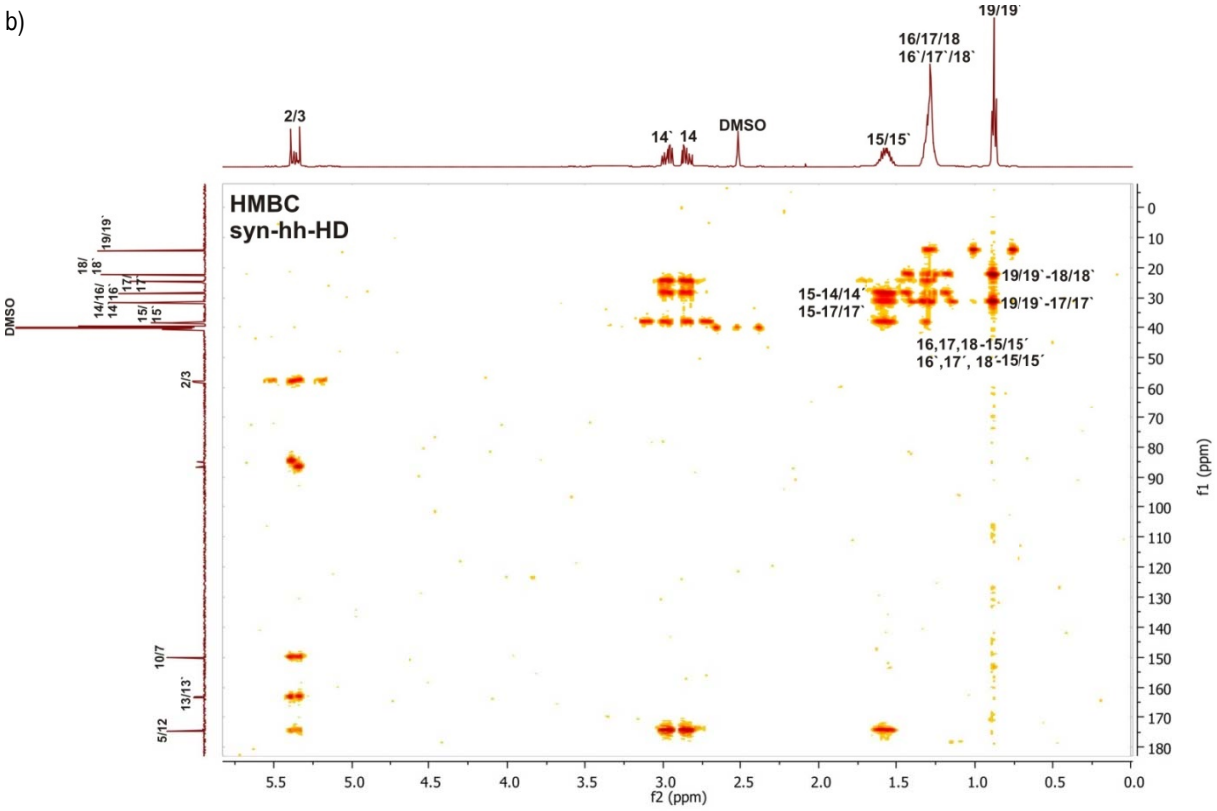
2D-NMR of H5FU-HD isomers

a)

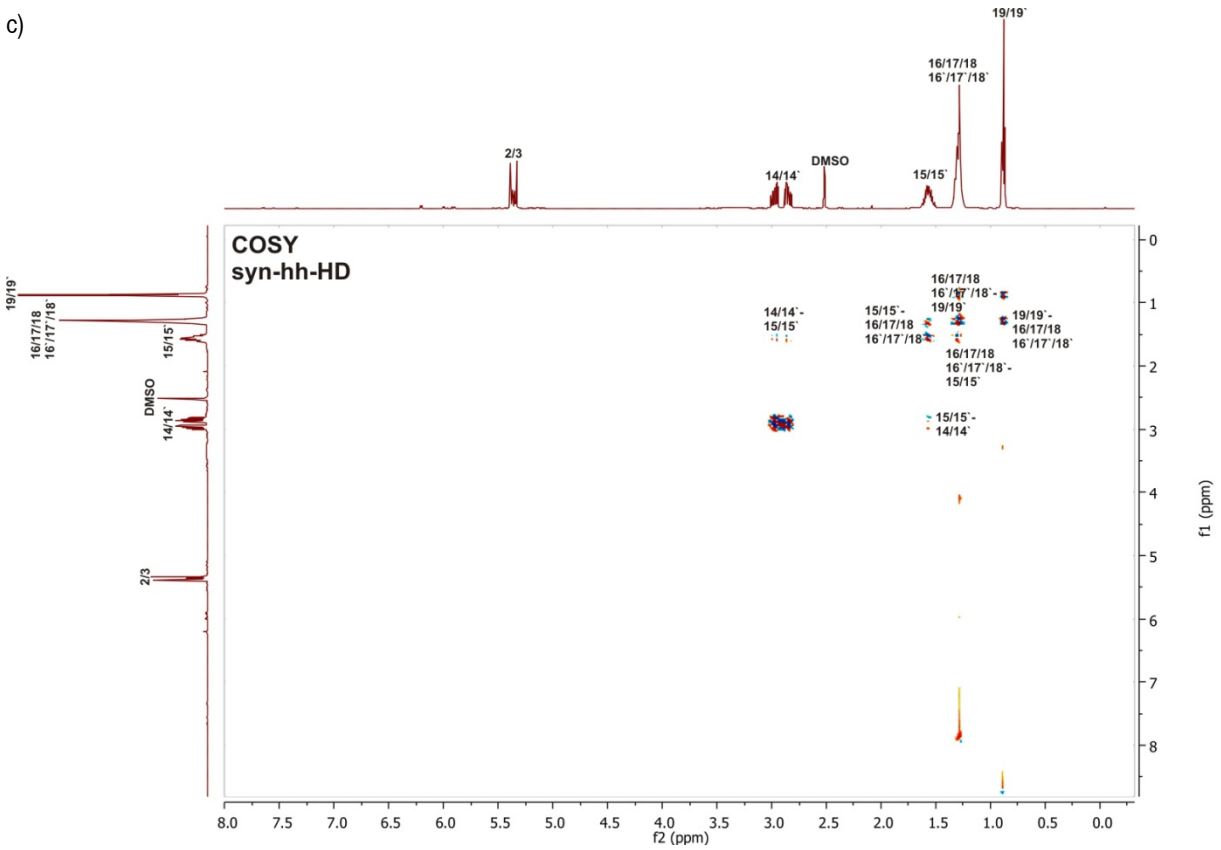



*syn*-hh-H5FU-HD

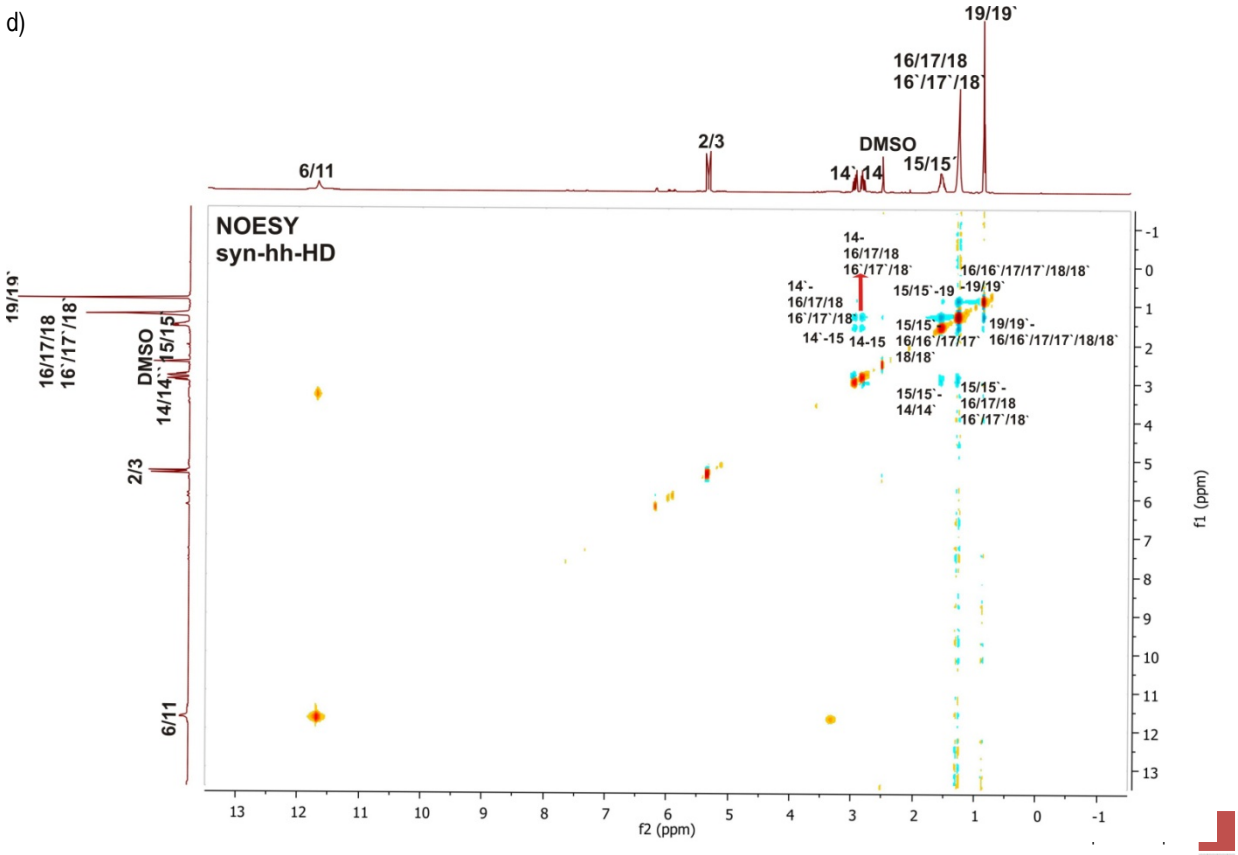
b)



c)


COSY
syn-hh-HD
19/19`
16/17/18
16`/17`/18`
2/3
14/14`
DMSO
15/15`
14`-
15/15`
15/15`-
16/17/18
16`/17`/18`
19/19`-
16/17/18
16`/17`/18`
16/17/18
16`/17`/18`-
15/15`
15/15`-
14/14`
f2 (ppm)
f1 (ppm)

d)


NOESY
syn-hh-HD
19/19`
16/17/18
16`/17`/18`
6/11
2/3
DMSO
14`
14
15/15`
14-
16/17/18
16`/17`/18`
16/16`/17/17`/18/18`
15/15`-19
-19/19`
14`-
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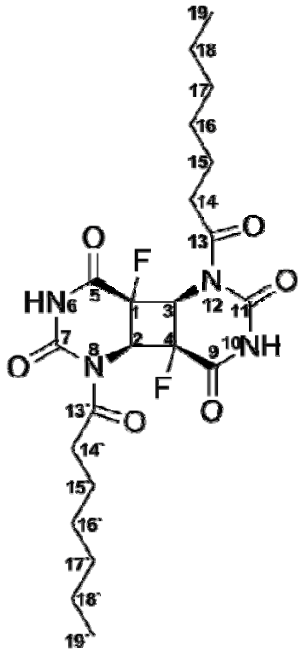
16/16`/17/17`/18/18`
15/15`-
14/14`
15/15`-
16/17/18
16`/17`/18`
f2 (ppm)
f1 (ppm)

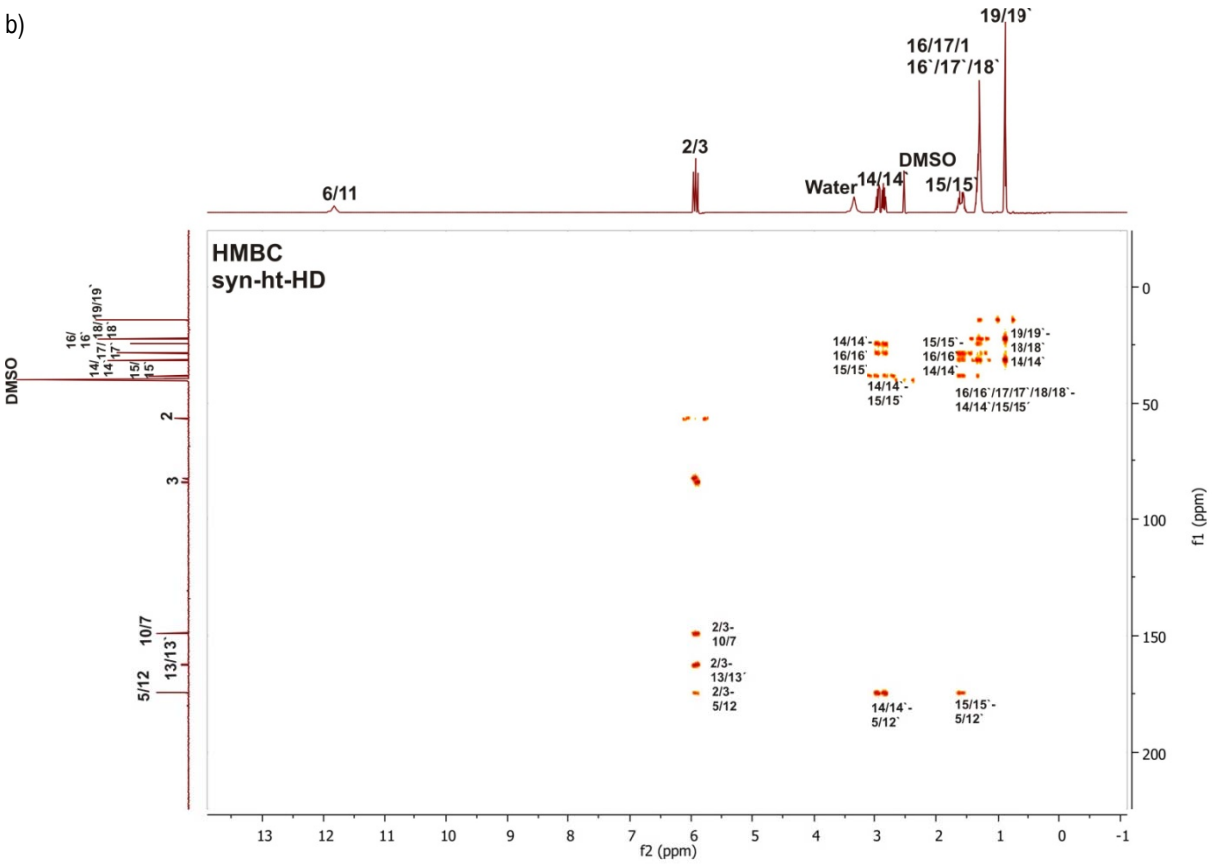
**Figure S2:** 2D-NMR of *syn*-hh-H5FU-HD for conformational assignment.(a) Structural formula, (b) HMBC, (c) COSY,(d) NOESY.

a)

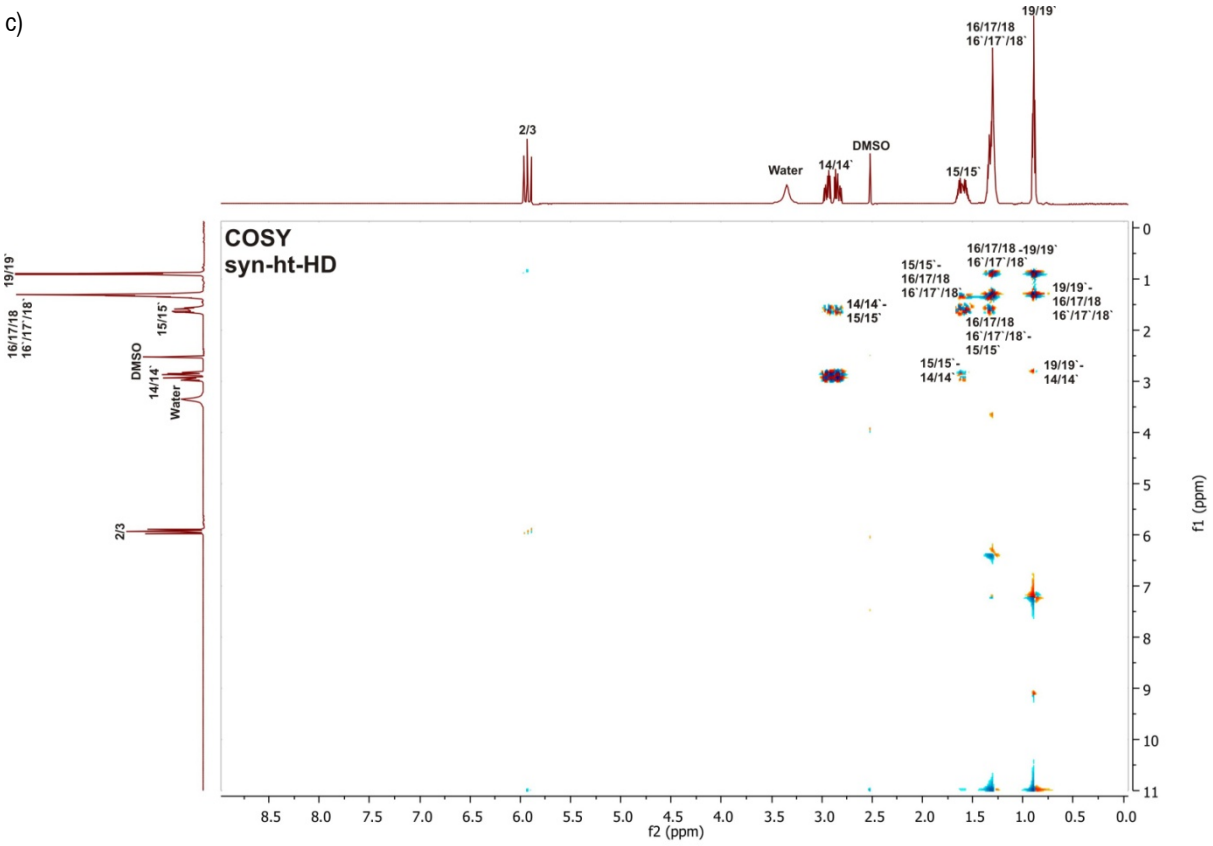



*syn*-ht-H5FU-HD

b)



c)


COSY
syn-ht-HD
19/19`
16/17/18
16`/17`/18`
15/15`
DMSO
14/14`
Water
2/3
f2 (ppm)
f1 (ppm)

d)

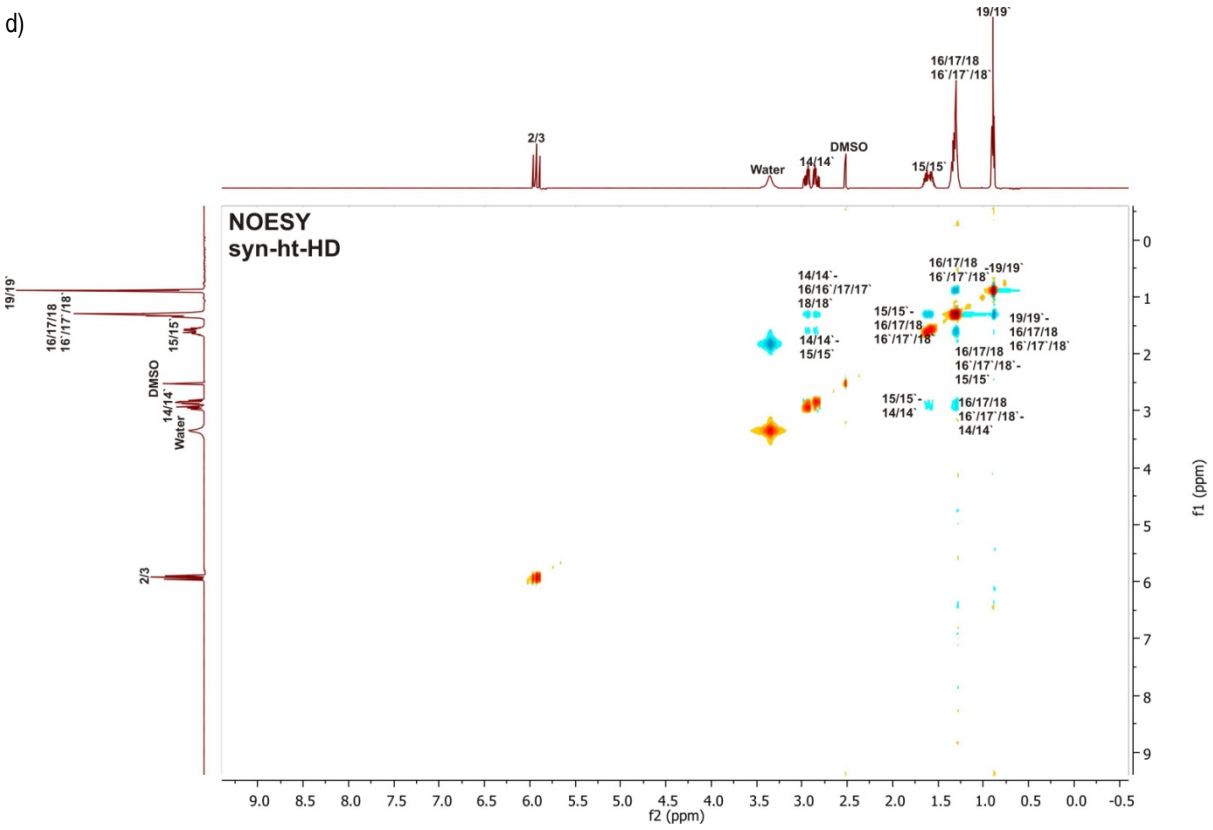
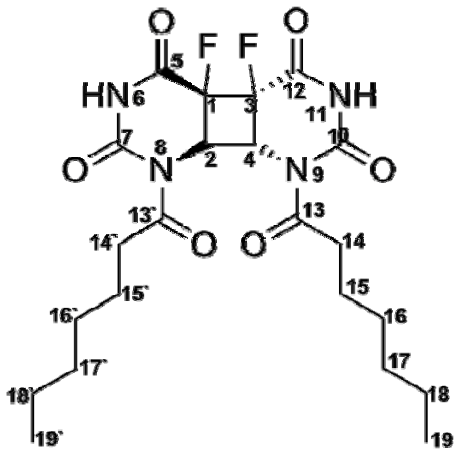

NOESY
syn-ht-HD
19/19`
16/17/18
16`/17`/18`
15/15`
DMSO
14/14`
Water
2/3
f2 (ppm)
f1 (ppm)

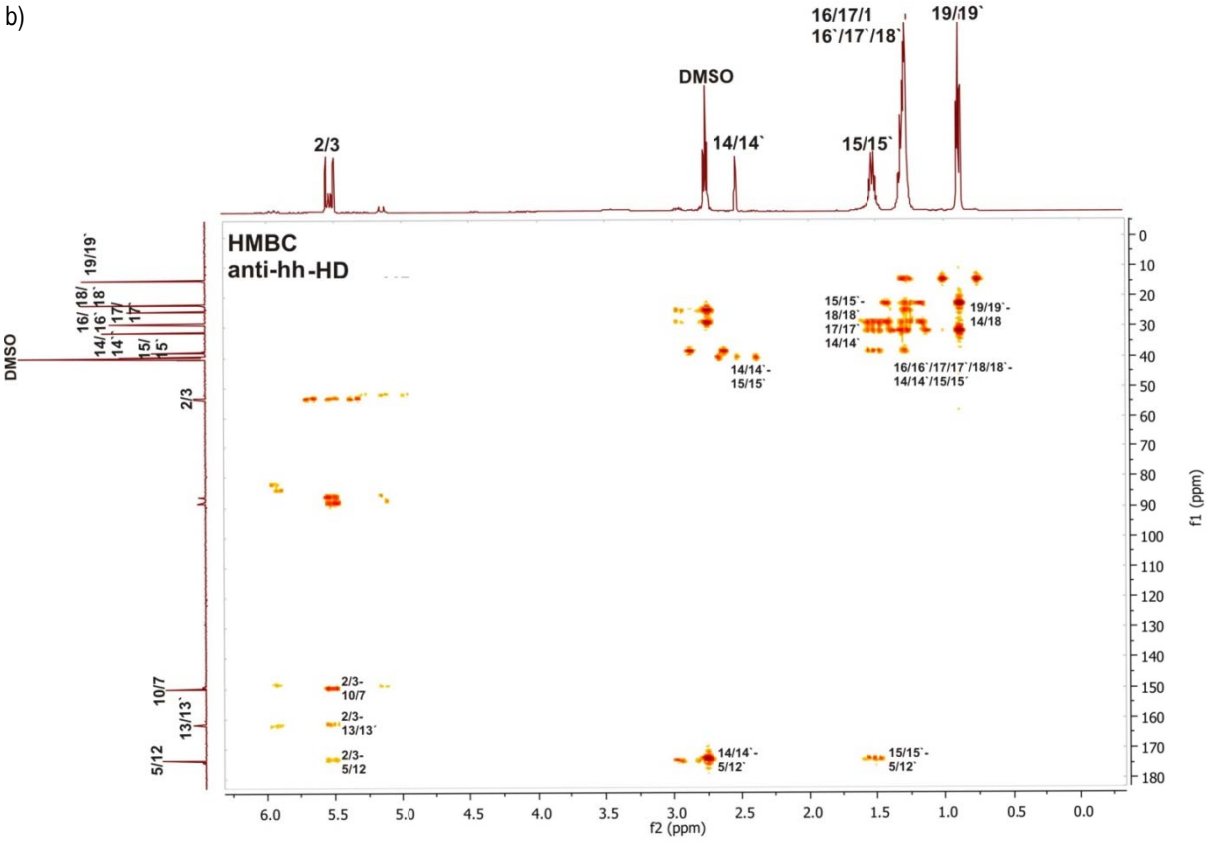
**Figure S3:** 2D-NMR of *syn*-ht-H5FU-HD for conformational assignment.(a) Structural formula, (b) HMBC, (c) COSY,(d) NOESY.

a)




*anti*-hh-H5FU-HD

b)



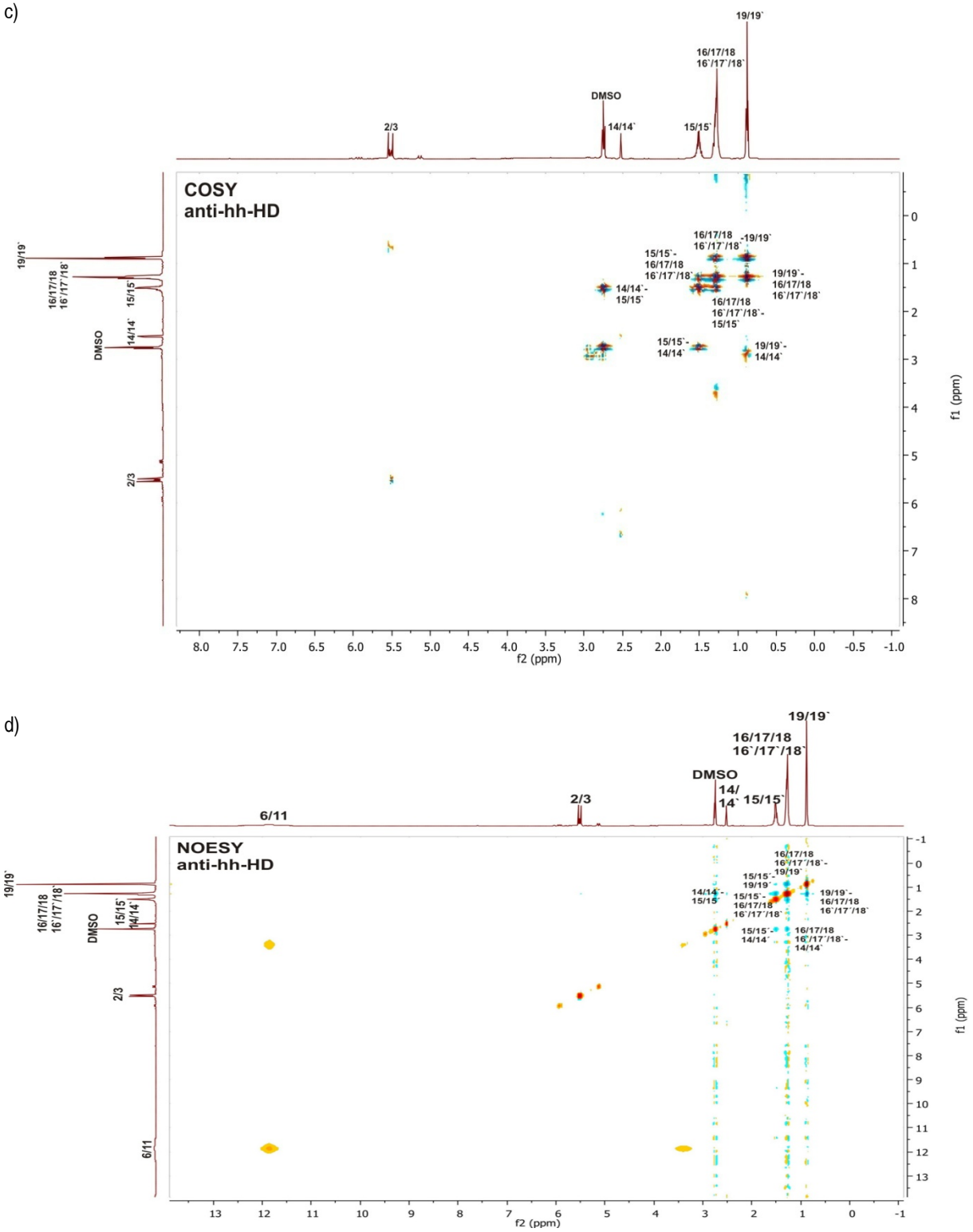



**Figure S4:** 2D-NMR of *anti*-hh-H5FU-HD for conformational assignment.(a) Structural formula, (b) HMBC, (c) COSY,(d) NOESY.

## Thermal stability of polymer - steam pressure sterilization

Steam pressure sterilization of the hydrated polymers in 0.9% NaCl solution was done in glass vials in an autoclave at 121 C at 2 bar pressure (2540 EL, Tuttnauer).

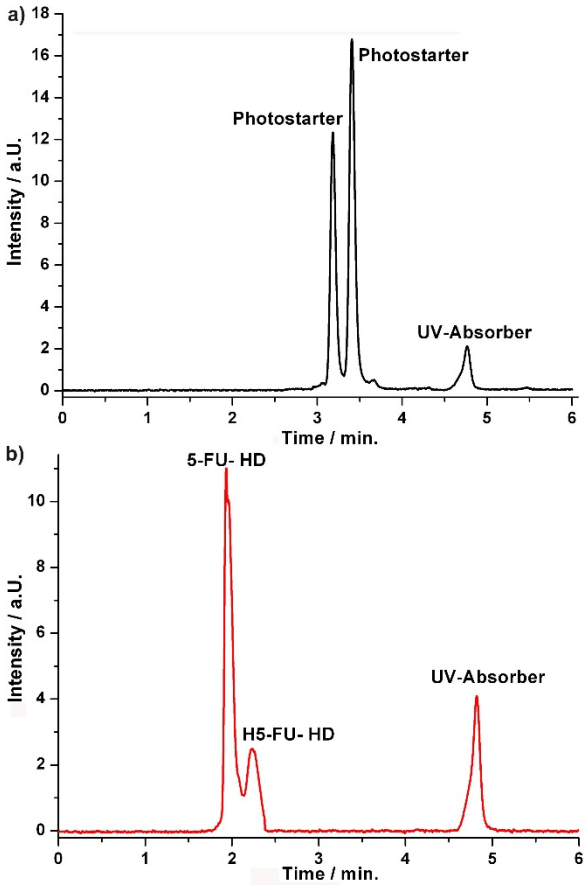



**Figure S5:** Sterilization of polymer samples at 121 C and 2 bar. HPLC chromatogram of the surrounding water solution after autoclaving process. (a) Chromatogram shows *syn*-hh-5FU-HD polymer, photochemical copolymerized, and (b) shows 5FU-HD matrix-embedded into polymer.

From the polymers only the two photostarter molecules as well as some non-polymerized UV-absorber is found in the solution (Fig. S5a). For means of comparison a polymer where the 5FU homodimer was just embedded into the polymer and not attached to the polymer moiety was co-analyzed. In this case free 5FU homodimer is observed in the sterilization solution as well as some UV-absorber.

## Analysis of photo-triggered released compounds

It was analysed by HPLC whether only 5FU was released upon photochemical excitation or any other compounds or debris. Only 5-FU was observed for SPA as well as TPA.

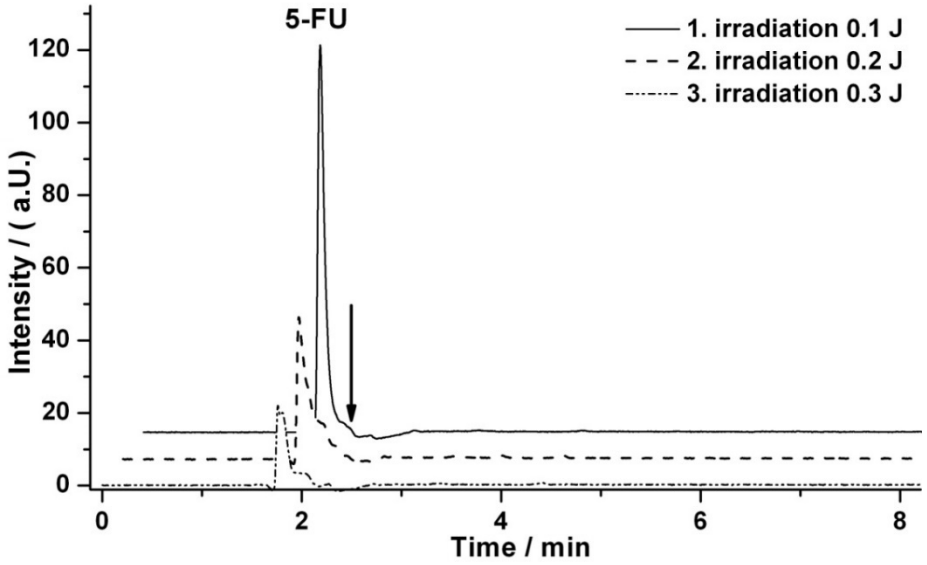



**Figure S7.**HPLC chromatograms of the drug release by consecutive TPA irradiations(Fig. 6b) with 532 nm pulsed laser. No other products except 5FU are released.