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Two-photon absorption triggered drug delivery from a polymer for intraocular lenses in presence of an UV-absorber

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ABSTRACT

Two-photon-absorption (TPA) triggered photochemistry is a versatile tool to photochemically release compounds in an uncaging reaction where the desired reaction should occur behind an UV absorbing barrier, e.g. as in intraocular lenses (IOLs). Nonlinear effects of the UV-absorbing barrier, in this case the cornea, are negligible as long as the laser light passes through it at an intensity low enough that nonlinear effects do not occur. Only in the focus of the beam, i.e. inside the IOL, the required intensities are reached and TPA triggered photo cleavage occurs. The situation becomes complicated as soon as UV-absorbers are admixed to the polymer material. We show that typical concentrations of an UV-absorber only slightly affect the TPA-triggered uncaging reaction rate. As a testbed we used a newly synthetized acrylic polymer derivatized with an *o*-nitrobenzyl linker group carrying 5-fluorouracil as the model drug to be released. No photochemical decomposition of the UV absorber was observed. The release rate of 5-fluorouracil in presence of the UV absorber was reduced by about 6% only. Further the polymer presented here, is the first to release unmodified 5-fluorouracil without any auxiliary groups attached. This is important for potential applications in humans.

1. Introduction

The photoactivity of *ortho*-nitrobenzyl compounds was described first by Silber and Ciamician [1]. In the 1960s they were introduced as photolabile protective groups into organic synthesis [2–4]. The concept of caged compounds, meaning the deactivation of biological compounds by derivatization with this photolabile protective group, was introduced in the late 1970s [5,6]. Today *ortho*-nitrobenzyl based caged compounds are used mainly for biophysical and biochemical investigations [7–10]. The mechanism of the single photon absorption (SPA) induced photo release process (Fig. 1) is quite well understood, as is the influence of additional substituents [11–15]. The ability to release a therapeutic payload from a carrier system is the major goal of drug delivery.

Our interest in photochemical drug delivery is focused on intraocular lenses (IOLs) which are implanted into the eye about 800-thousand times per year in Germany alone. Most often the cause is cataract, which according to the WHO is responsible of 33% of visual impairment worldwide [16]. Triggered drug release from implanted IOLs is a novel approach to treat secondary cataract, a common post-operative complication occurring in 10–50% of the cases within 3–5 years [17,18]. Secondary cataract is an opacification caused by the proliferation and the migration of retained lens epithelial cells into the visual axis. Several photoactive materials have been developed to provide photochemical drug delivery from IOLs, matching the required material properties like transparency, two-photon activity and optimized drug diffusion. In all these systems drug attachment to the polymer moiety is achieved through [2+2] cycloaddition. This photochemical step in the synthesis of the materials causes low yields or extensive purification steps [19–26].

In technical applications of acrylic polymers UV absorbers are integral compounds of the polymers on the market. The lightfastness of acrylic polymers is dramatically decreased without such additives and tanning is observed even after exposure to small UV light doses. For intraocular lenses the UV absorber requirement is regulated in order to protect the posterior of the eye from UV exposure.

As both UV absorber and photo cleavable drug-linker are uniformly distributed within the bulk polymer there is a potential conflict between the desired therapeutic photo cleavage of the

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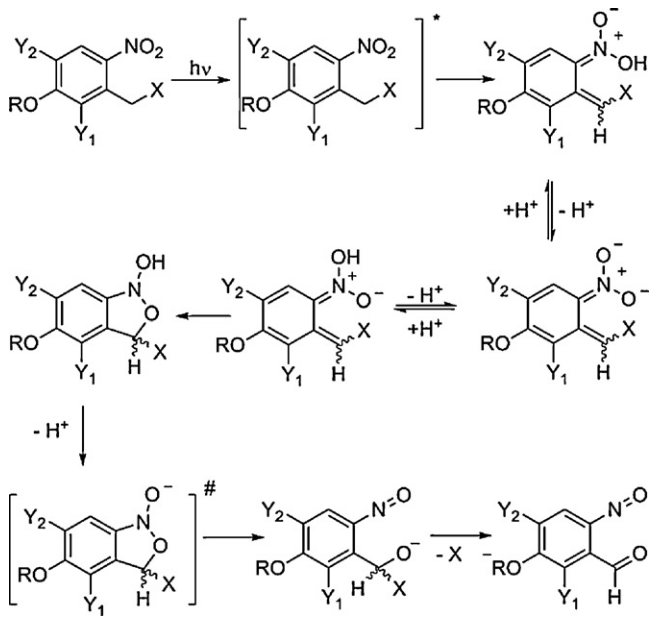

Fig. 1. General mechanism of the photolysis of *ortho*-nitrobenzyl compounds: X = released compound, Y_1 = additional nitro group, Y_2 = electron donating group (e.g. OMe), OR = possible linkage (e.g. to polymer).

drug from the polymer and an undesired decomposition of the UV-absorber. Further the UV absorber may diminish or even suppress the two-photon controlled release of drug from the polymer.

We addressed this problem and found that a suitably selected UV absorber is nicely compatible with the two-photon absorption triggered drug release from the polymer. As a testbed we used an *ortho*-nitrobenzyl group as a linker, 5-fluorouracil as a model drug, and 2(4-benzoyl-3-hydroxy phenoxy) ethyl acrylate (BHP-EA) as an UV absorber. 5-FU was chosen, because it is able to address the secondary cataract inducing proliferating epithelia cells and its low therapeutical dosage enables the storage of enough drug within a single IOL. We have prepared IOLs from this newly synthesized polymer and analyzed the drug release function under two-photon excitation.

Only very few publications deal with TPA of *ortho*-nitrobenzyl compounds. In 2003 Diaspro et al. investigated the two-photon induced properties of 2-nitrobenzaldehyde as a caged proton compound utilizing fluorescent labeled nanocapsules as a new sensor [27]. Three years later Aujard et al. published the uncaging cross-sections for one and two-photon excitation of *ortho*-nitrobenzyl based protecting groups with red-shifted absorptions [28]. Recently Banerjee et al. reported a multifunctional magnetic nanocarrier which enables the release of a drug conjugated by an *ortho*-nitrobenzyl moiety by absorption of near infrared irradiation due to up-conversion of the irradiated energy [29]. Two years ago Agasti et al. reported the photochemical drug release of 5FU from gold nanoparticles utilizing a nitrobenzyl moiety similar to the synthesized monomer in this work [30]. However such materials are intensely colored and may not be used in applications like intraocular lenses.

To our knowledge, this is the first report on two-photon absorption induced drug release in presence of an UV-absorber from an optically clear and more or less colorless polymer using an *ortho*-nitrobenzyl moiety as the photo cleavable linker where the drug is released without any auxiliary groups attached.

2. Experimental

2.1. Materials and methods

All chemicals used were purchased from Sigma–Aldrich (precursors for **2** and **3**), Acros Scientific, Merck (TLC plates), ABCR (PPH_3), Fluorochem (TBS-Cl), TCI Europe (DIAD), or Melrob (MAA) and were used as received. Anhydrous solvents were purchased from Sigma–Aldrich or Acros Scientific and used as received. All reactions were done under Schlenk-conditions with argon 4.8 as protective gas purchased from Air Liquide. The reactions were monitored and characterized by thin layer chromatography (Merck, TLC silica gel 60 F_{254} aluminum sheets), 300 MHz ^{1}H NMR, 282 MHz ^{19}F NMR and 75 MHz ^{13}C NMR spectra (Bruker, Advance 300 spectrometer). Single photon absorption in solution was induced with a Shimadzu RF-1502 spectrometer using an excitation wavelength of 266 nm. The light energy exposed to the samples was determined by using azobenzene as an actinometer following Gauglitz and Hubig [31]. Irradiation at 532 nm was done utilizing an Infinity 40–100 (Coherent Inc.), a q-switched Nd:YAG laser with a pulse length of 3 ns and a repetition rate of 40 Hz. The beam has a flat top profile and the diameter was adjusted to 5 mm by an aperture. The energy at the location of the sample was measured using a Fieldmaster GS (Coherent) set to the appropriate wavelength and equipped with a power meter head Model 80 (Coherent). Analytical HPLC was performed on a Dionex Ultimate 3000 equipped with a Nucleosil RP18 (3 μm Bischoff) column utilizing mixtures of acetonitrile (ACN) (Rotisolv HPLC grade; Roth) and deionized water (purified by a TKA-Micro ultra-pure water purification system (0.06 μS) and acidified with 300 μl l^{-1} phosphoric acid) as eluent. Peak detection was enabled by diode array detector (DAD). Quantitative measurements were done by peak integration and referred to calibration standards of the compounds examined. All UV–vis-spectra were recorded on a Lambda 35 spectrometer (Perkin Elmer) between 190 nm and 700 nm with 480 nm min^{-1} in steps of 1.0 nm. Measurements in solution were performed in quartz cuvettes (QS, Hellma; UQ, Portman Instruments) with 10 mm path length in the given solvents.

2.2. Synthetic procedures

3-Hydroxy-4-methoxy-2,6-dinitrobenzaldehyde **(2)** and 3-hydroxy-4-methoxy-2,6-dinitrobenzyl alcohol **(3)** as well as 5-iodo-1-*tert*-butyldimethylsilyloxypentan and 1-*N*-benzoyl-5-fluorouracil (Bz5FU) were synthesized following literature procedures [30,32–34].

2.2.1. 3-(5-(Tert-butyldimethylsilyloxy)pentyloxy)-4-methoxy-2,6-dinitrobenzylalcohol ***(4)***

5 mmol of **3** and 25 mmol of potassium carbonate were suspended in 50 ml of *N*,*N*-dimethylformamide (DMF). After addition of 5.5 mmol of the alcylhalide the suspension was heated to 70 °C for 48 h. After cooling to room temperature (RT) all insoluble salts were filtered off. The reaction solution was diluted with 150 ml of deionized water and extracted 3-times with ethyl acetate. The combined organic layers were washed with brine and dried over anhydrous sodium sulfate. Evaporation of the solvent yielded the crude product which was further purified by column chromatography on silica gel with pentane and ethyl acetate (4:1) as eluent. 1.26 g, 2.9 mmol, 57% yield: ^{1}H NMR result (300 MHz, $CDCl_3$): δ/ppm = 7.70 (s, 1H, $C_{ar}H$), 4.70 (d, 2H, ^3J = 7.3 Hz, C_{ar}—CH_2—OH), 4.25 (t, 2H, ^3J = 6.6 Hz, C_{ar}—O—CH_2—CH_2), 3.99 (s, 3H, O—CH_3), 3.62 (t, 2H, ^3J = 6.3 Hz, CH_2–OTBS), 1.79–1.69 (m, 2H, CH_2 aliphatic), 1.61–1.51 (m, 4H, CH_2 aliphatic), 0.89 (s, 9H, Si—C—$(CH_3)_3$), 0.05 (s, 6H, Si—$(CH_3)_2$). ^{13}C NMR result (75 MHz, $CDCl_3$): δ/ppm = 152.46, 144.62, 121.27, 110.28, 75.48, 62.92, 57.07, 56.84, 32.32, 29.61,

25.94, 25.62, 21.83, 18.33, 1.00, −5.31. HRMS (ESI): m/z = 445.2008 [M+H$^+$] Δmmu = 0.7

2.2.2. 3-N-(3-(5-(Tert-butyldimethylsilyloxy)pentyloxy)-4-methoxy-2,6-dinitrobenzyl)-1-N-benzoyl-5-fluoro-uracil ***5***

At 0 °C 14 mol of diisopropyl azodicarboxylate (DIAD) was added slowly to a suspension of 14 mmol of triphenylphosphine (PPh_3) in 40 ml anhydrous tetrahydrofuran (THF) and stirred for additional 30 min. 20 ml (7 mmol) of this preformed beige complex suspension was added slowly to a suspension of 7 mmol Bz5FU and 7 mol of **4** in 20 ml anhydrous THF at −20 °C. The reaction mixture was stirred for 15 h and allowed to warm to RT. After evaporation of the solvent the crude reaction mixture was purified by column chromatography on silica gel with pentane and *tert*-butylmethylether (1:1) as eluent. 3.7 g, 5.6 mmol, 80% yield: ^{1}H NMR result (300 MHz, $CDCl_3$): δ/ppm = 7.95 (d, 2H, ^3J = 7.3 Hz, Bz: C_q—C*H*—CH) 7.70–7.65 (m, 2H, C_{ar}*H*—C—NO_2, Bz: C_q—CH—C*H*), 7.52 (t, 2H, ^3J = 7.3 Hz, Bz: CH—CH—C*H*), 7.28–7.26 (m, C*H*=CF, solvent signal), 5.07 (s, 2H, C_{ar}—CH_2—N), 4.29 (t, 2H, ^3J = 6.6 Hz, C_{ar}—O—CH_2—CH_2), 4.02 (s, 3H, O—CH_3), 3.63 (t, 2H, ^3J = 6.3 Hz, CH_2–OTBS), 1.81–1.72 (m, 2H, CH_2 aliphatic), 1.61–1.51 (m, 4H, CH_2 aliphatic), 0.90 (s, 9H, Si—C—(CH_3)$_3$), 0.06 (s, 6H, Si—(CH_3)$_2$). ^{19}F NMR result (282 MHz, $CDCl_3$): δ/ppm = −163.4. ^{13}C NMR result (75 MHz, $CDCl_3$): δ/ppm = 166.62, 153.57, 148.13, 144.86, 144.26, 138.53, 135.45, 130.75, 130.51, 129.25, 127.22, 126.77, 113.87, 110.58, 75.79, 62.88, 57.02, 44.24, 32.31, 32.10, 29.61, 28.60, 25.94, 22.78, 21.88, 18.34, 1.00, −5.30. HRMS (ESI): m/z = 683.2155 [M+Na$^+$] Δmmu = −0.1

2.2.3. 3-N-(3-(5-Methacryloxypentyloxy)-4-methoxy-2,6-dinitrobenzyl)-5-fluorouracil ***6***

To 3 mmol of **5** dissolved in 15 ml methanol 15 ml of concentrated hydrochloric acid was added. The resulting solution was stirred at 60 °C for 15 h. After cooling to RT the reaction mixture was diluted with 50 ml of deionized water and extracted with chloroform for three times. The combined organic layers were dried over anhydrous sodium sulfate and the solvent was evaporated under reduced pressure. The sustained crude product was purified by column chromatography on silica gel with chloroform and methanol (15:1) as eluent. At 0 °C 2.75 mmol (556 mg) of DIAD was added slowly to a suspension of 2.75 mmol (710 mg) of PPh_3 in anhydrous THF and stirred for additional 30 min. This preformed beige complex suspension was added slowly to a suspension of 2.75 mmol (233.2 μl) of methacrylic acid (MAA) and 2.5 mmol of deprotected **5** in anhydrous THF at −20 °C. The reaction mixture was stirred for 3 h and allowed to warm to RT. After evaporation of the solvent the crude reaction mixture was purified by column chromatography on silica gel with pentane and ethyl acetate (3:2) as eluent resulting in 702 mg (1.375 mmol, 55% yield) of white solid. H NMR result (300 MHz, $CDCl_3$): δ/ppm = 7.70 (s, 1H, C_{ar}*H*), 7.18 (d, 1H, J = 5.7 Hz, C*H*=CF), 6.11 (m, 1H, CH_2=C_q), 5.56 (p, 1H, J = 1.5 Hz, CH_2=C_q), 5.04 (s, 2H, C_{ar}—CH_2—N), 4.28 (t, 2H, ^3J = 6.4 Hz, C_{ar}—O—CH_2—CH_2), 4.16 (t, 2H, ^3J = 6.5 Hz, CH_2—O—(C=O)), 4.02 (s, 3H, O—CH_3), 1.94 (m, 3H, CH_2=C_q—CH_3), 1.82–1.68 (m, 4H, CH_2 aliphatic), 1.53–1.46 (m, 2H, CH_2 aliphatic). ^{19}F NMR result (282 MHz, $CDCl_3$): δ/ppm = −164.8. ^{13}C NMR result (75 MHz, $CDCl_3$): δ/ppm = 153.49, 148.95, 146.82, 144.60, 144.45, 138.86, 136.41, 129.53, 128.33, 127.65, 127.20, 125.33, 113.96, 110.51, 75.42, 64.35, 57.03, 43.97, 29.41, 28.20, 22.09, 18.28. HRMS (ESI): m/z = 533.1287 [M+Na$^+$] Δmmu = −0.3.

3. Results and discussion

3.1. Synthesis of drug loaded monomer

Synthesis started from isovanilin, which was nitrated in 2- and 6-position by concentrated nitric acid. After reduction of the aldehyde with sodium borohydride [30] *tert*-butyldimethyl protected pentanol was introduced at the more reactive phenolic alcohol in 3-position utilizing Williamson' ether synthesis. In the second step 5FU was coupled to the precursor (**4**) using the Mitsonobu reaction to form the desired amine bond. This synthesis was adopted from the former reported Mitsonobu coupling of *N*-3-benzoylthymine with various alcohols [35] and increased the yield of the deprotected drug loaded precursor from 5% over four steps [30] to 40% over three steps and lowered the reaction time from 73 h to 33 h. Finally **5** was deprotected and esterified with methacrylic acid to yield a monomer suitable for photo chemical drug delivery (**6**) which was copolymerized with a MMA/HEMA mixture (Fig. 2).

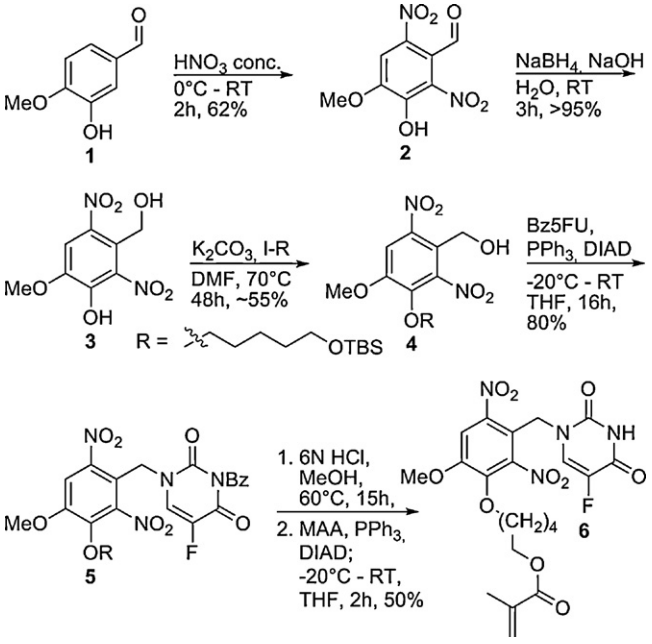

Fig. 2. Synthesis of 5FU loaded photoactive monomer.

3.2. Photochemical characterization—selection of UV-absorber

Fig. 3 shows the absorption spectra of **4**, **5** and **6** as well as the spectrum of the UV-absorber BHP-EA at a concentration of 0.03 mM. The *ortho*-nitrobenzyl precursor **4** has a weak broad absorption between 260 nm and 400 nm with a maximum at 324 nm. After functionalization with benzoyl-5-fluorouracil (Bz5FU) (**5**), a stronger absorption band with a maximum at 252 nm

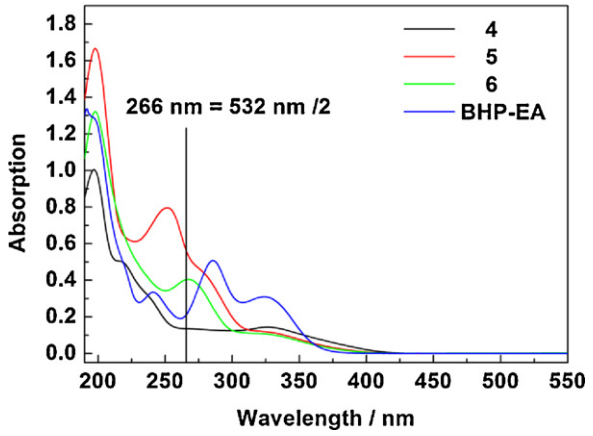

Fig. 3. Absorption spectra of 0.03 mM solutions of **4**, **5** and **6** in ACN.

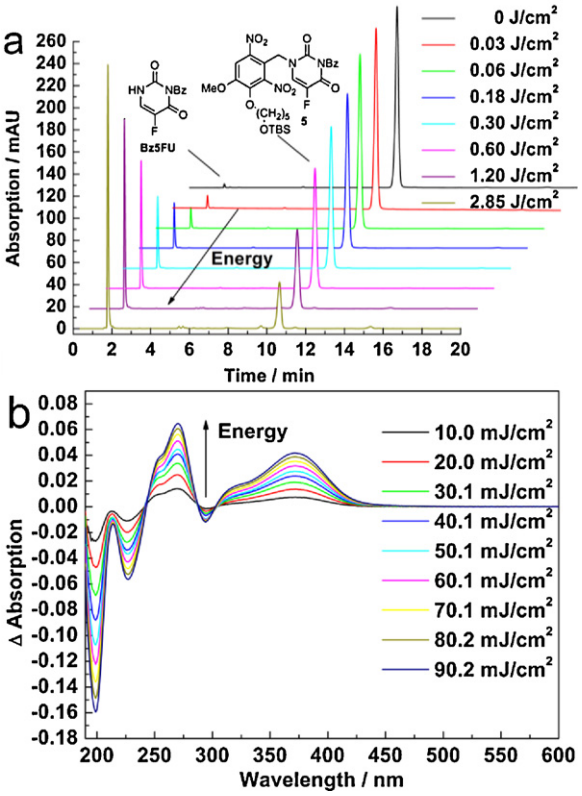

Fig. 4. (a) HPLC chromatograms of a 1 mM solution of **5** after consecutive irradiations with 266 nm light with total energies given. (b) Change in absorption after irradiation with 266 nm (total energies given) of a 25 μM solution of **5** in ACN.

is observed. Deprotection of 5FU and esterification with MAA shifts the absorption band of the monomer **6** to 268 nm which corresponds well to the main absorption of the unfunctionalized free drug. Irradiation with 266 nm, i.e. single photon absorption, or with 532 nm for the TPA process results in excitation of the molecule near the maximum of the absorption of 5FU. It is likely that the majority of the energy gets absorbed by the uracil part and then to be transferred to the nitrobenzyl moiety. Longer lifetimes for nucleobase-compounds have been measured by Kohler et al., which would make such an energy transfer supposable [36]. Steiner et al. have investigated comparable substances and could show that the energy transfer from a linked chromophor to the nitrobenzyl part was around double figure ns. But conclusively the H-atom transfer of the uncaging mechanism seems to determine the lifetime of such combined chromophors [37,38]. A more detailed analysis can only come from transient absorption measurements. But the absorption of **6** corresponds quite nicely with the chosen UV-absorber BHP-AE, which is an efficent UV-blocker in the range between 275 nm and 350 nm. At a wavelength of 266 nm it exhibits a minimum in the spectrum preserving the possiblity of TPA induced drug release, assuming that BHP-AE remains uneffected by the irradiated pulsed laser light. This was checked by irradiation of 3 mM solution of BHP-AE with 25.5 kJ of laser light at 532 nm. Neither in the HPLC chromatogram nor in the UV–vis spectra of the irraditated solution any change in retention time or absorbance was monitored.

3.3. Single photon induced drug release in solution

In order to avoid undesired side reactions the protected monomer precursor **5**, which has the same chromophor structure as the reactive monomer **6**, was characterized in its photochemical properties in solution. Fig. 4a shows HPLC chromatograms of

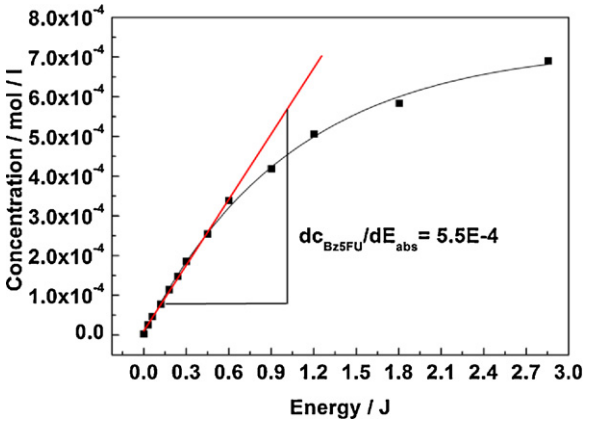

Fig. 5. Determination of quantum efficiency from the change in concentration versus the absorbed energy for **5**.

a 1 mM solution of **5** after consecutive irradiations at 266 nm. The increasing signal at a retention time of 1.8 min corresponds to the released Bz5FU, the decreasing signal at 10.7 min correspond to cleaved **5** and the nitroso-aldehyde generated during the photo induced release process. They have the same retention time but can be identified due to their different absorption spectra. To quantify the degree of cleavage the peak area of Bz5FU was recorded and correlated to Bz5FU standards of known concentration.

Fig. 4b depicts the disassembly of the caging compound in the spectral range of 190–250 nm. The range 250–305 nm stands for the buildup of secluded drug molecules, whereas the region beyond that represents the formation of the nitroso-compound. The isosbestic points at 250, 280 and 305 nm, together with the tidy chromatogram from Fig. 4a stand for a clean uncaging reaction.

The efficiency of the photo cleavage at low conversion in the beginning of the reaction is quantified by the single photon quantum yield φ_{cleave}

$$\varphi_{\text{cleave}} = \frac{n_{\text{drug}}}{n_{\text{photon}}} = \frac{n_{\text{drug}}}{E_{\text{abs}}} \cdot \frac{E_{\text{abs}}}{n_{\text{photon}}} \quad \text{(I)}$$

n_{drug} represents the number of cleaved molecules, n_{photon} the number of photons absorbed and E_{abs} the absorbed energy. The number of cleaved molecules per absorbed energy can be calculated by

$$\frac{n_{\text{drug}}}{E_{\text{abs}}} = \frac{c_{\text{drug}}}{E_{\text{abs}}} \cdot V \cdot N_A \quad \text{(II)}$$

with c_{drug} being the concentration of cleaved molecules, V the volume of the probe (1 ml) and N_A the Avogadro constant. Plotting the concentration of cleaved dimers versus the actual energy absorbed (Fig. 5) results in a linear increase with $c_{\text{drug}} \cdot E_{\text{abs}}^{-1}$ as the slope of the linear fitting of the data points. With (II) $n_{\text{drug}} \cdot E_{\text{abs}}^{-1}$ is calculated to be $3.64 \times 10^{17}\ \text{J}^{-1}$. The number of photons per energy unit results in $1.34 \times 10^{18}\ \text{J}^{-1}$ according to (III)

$$\frac{n_{\text{photon}}}{E_{\text{abs}}} = \frac{\lambda}{c \cdot h} \quad \text{(III)}$$

λ represents the wavelength, c the speed of light and h the Planck constant. Using (I) the quantum yield of the cleavage for **5** is determined to be 27% corresponding well with the literature [39].

3.4. Two-photon induced drug release in solution

To verify that the drug release is possible in a two-photon absorption triggered process a 0.2 mM solution of **5** in ACN was irradiated with 532 nm at different pulse energies over a constant time. Fig. 6a shows the change in absorption at 370 nm, which is proportional to the drug release versus the irradiation time at

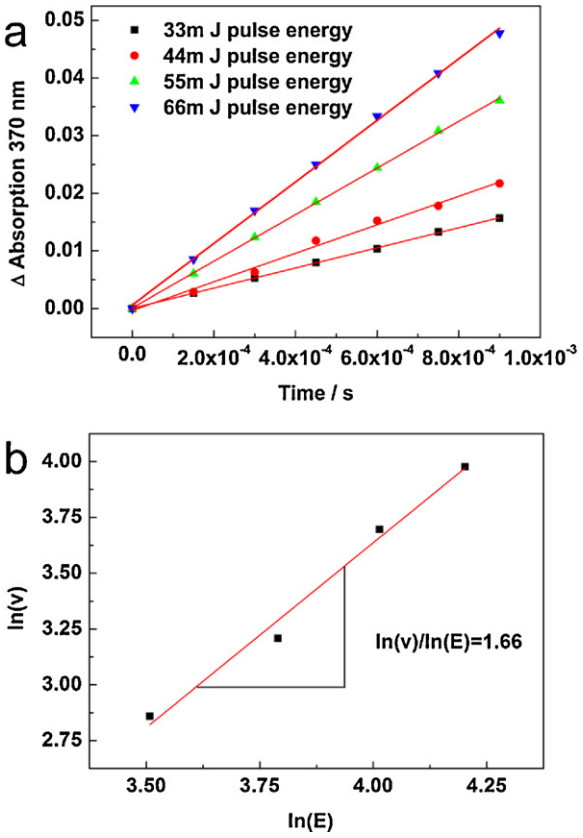

Fig. 6. (a) Absorption at 370 nm versus time of irradiation at different pulse energies and (b) double logarithmic plot of reaction rate over pulse energy.

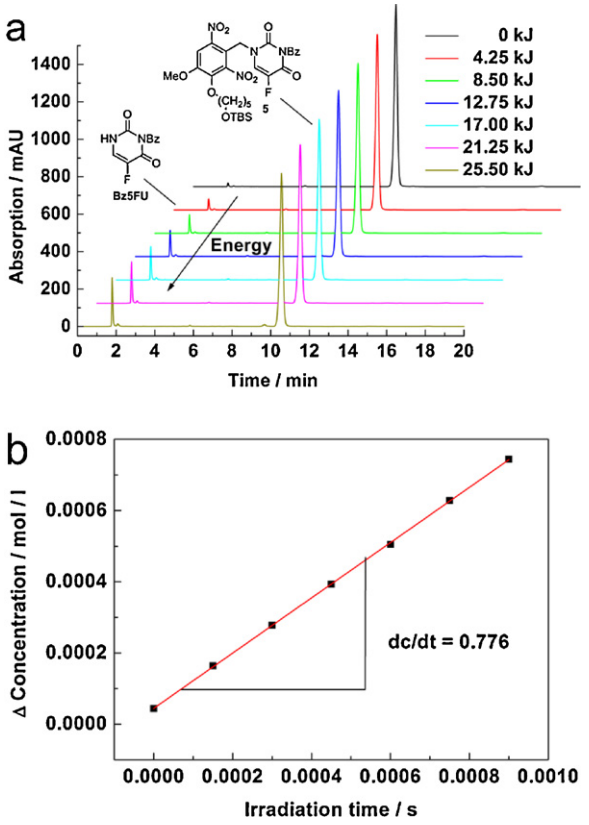

Fig. 7. (a) HPLC chromatograms of a 6.05 mM solution of **5** after consecutive irradiations with 532 nm light pulses with the total energies given. (b) Change in concentration of released Bz5FU over irradiation time.

different pulse energies. Because the efficacy of the TPA-induced excitation depends on the square of the light intensity, the drug release is expected to be proportional to the square of the applied laser intensity. Fig. 6b shows a double logarithmic plot with a slope of 1.66 indicating a TPA process. The deviation from a slope value of 2 could arise from non-linear absorption effects from the free drug molecule.

To quantify the TPA mediated drug release a 6.05 mM solution of **5** in ACN was irradiated at 532 nm with increasing energy doses. The photochemical release process was monitored by HPLC.

Again the increasing signal at a retention time of 1.8 min in Fig. 7a corresponds to the released Bz5FU, the decreasing signal at 10.7 min corresponds to cleaved **5** and the nitroso aldehyde generated during the photo-induced release process. The TPA cross section σ_{TPA} can be calculated from Eq. (IV)

$$\sigma_{TPA} = \frac{dc}{dt} \cdot V_{total} \cdot \left(\frac{h \cdot c \cdot A \cdot t}{E \cdot \lambda}\right)^2 \cdot \frac{1}{\Phi_{TPA} \cdot c_0 \cdot V_{ir}} \quad \text{(IV)}$$

With dc/dt corresponding to the change in concentration of released Bz5FU over time (Fig. 7b), V_{total} to the total sample volume (1.5 ml), E to the pulse energy, A to the irradiated area (0.196 cm^2), t to the pulse length (3 ns) Φ_{TPA} to the TPA quantum yield, c_0 to the starting concentration of the irradiated solution and V_{ir} to the irradiated volume (0.196 cm^3). Under the assumption that the quantum yield of the TPA is equal to the SPA quantum yield a TPA cross section of 2.41 GM can be obtained. This value exceeds the measured values of other *o*-NBnCs in the range of 0.01–0.1 GM [28] by more than one order of magnitude. This value is about the same compared to the TPA cross sections of photochemical drug delivery alternatives like coumarin or tetralone homo or hetero dimers [19,21].

The next step was to prove that the TPA triggered drug release is still possible in the presence of BHP-EA as the UV absorbing agent. For this purpose a mixture of 0.4 wt% (6.05 mM) of **5** and 0.1 wt% (3.2 mM) of BHP-EA corresponding to a 1 mm thick IOL with the common amount of 1 wt% of UV-absorber and a active drug load of 1 wt%, was irradiated in a 10 mm cuvette with pulsed laser light at 532 nm. Fig. 8a shows the HPLC chromatograms of the irradiated solution, as well as the amount of released Bz5FU over the irradiated energy compared to the achieved release from a solution without BHP-EA. The signal at a retention time of 3.2 min corresponds to BHP-EA and remains unaffected over the complete irradiation. As Fig. 8b shows the presence of BHP-AE lowers the total amount of released Bz5FU by 6% only.

3.5. Two-photon induced drug release from polymer

A mixture of 86.5 wt% HEMA, 12 wt% MMA, 1 wt% EGDMA as cross linker and a combination of 0.25 wt% of campherchinone and 0.25 wt% of ethyl-4-aminobenzoate as photo initiator at 465 nm served as basic monomer solution. Addition of 1 wt% of BHP-EA and 4 wt% of **6** to this solution resulted in the final polymerization mixture, corresponding to a net drug load of 1 wt% of 5FU. The resulting slightly yellow transparent plate (Fig. 9a) was extracted with distilled water over 3 days to remove the initiator and unreacted monomer. A maximum water uptake of about 22.8% was reached. TPA induced drug release from the polymer matrix could be achieved in two steps by irradiation of the polymer plate with 0.85 kJ each at 532 nm. The UV absorption at 268 nm of the surrounding water the polymer plate was immersed in, was monitored and the diffusion time and the final amount of released drug were determined (Fig. 9b). The diffusion time from the model polymer plate was very fast. A steady state 5FU concentration was reached within 10 min after the irradiation. The drug release results in an increasing absorption at 370 nm (for comparison see Fig. 4b). This

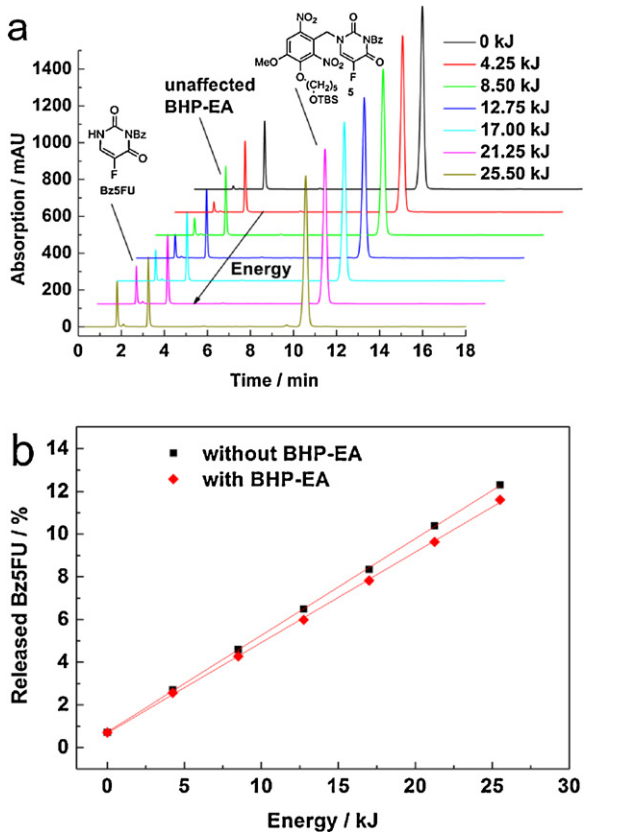

Fig. 8. (a) HPLC chromatograms of a 6.05 mM solution of **5** in the presence of BHP-EA after consecutive irradiations with 532 nm light with the total energies given. (b) Amount released Bz5FU with and without BHP-AE.

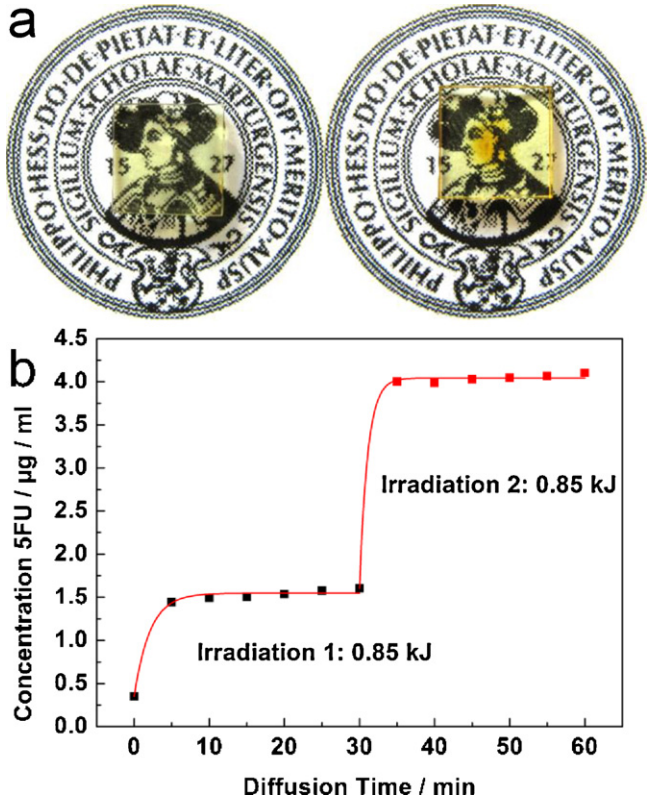

Fig. 9. (a) Drug loaded polymer plate before (left) and after irradiation at 532 nm with 0.85 kJ. (Right) (b) concentration of released 5FU from the polymer from two consecutive irradiation steps. (For interpretation of the references to color in text, the reader is referred to the web version of this article.)

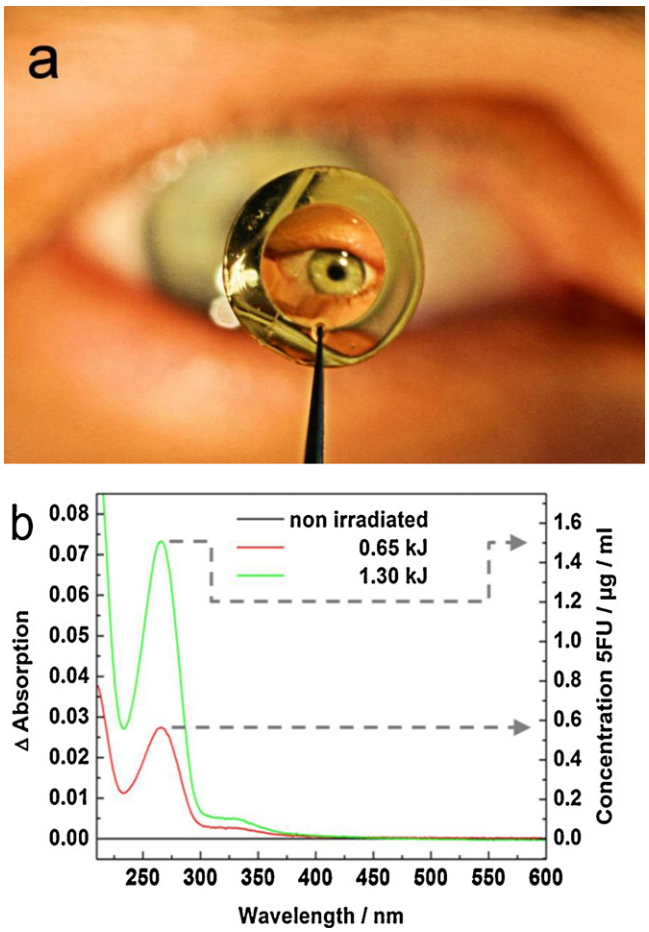

Fig. 10. (a) Prototype IOL in front of a human eye. (b) Absorption spectra of 5FU released by TPA from the IOL.

absorption band, corresponding to the generated nitroso functionality, unfortunately causes a slight increase in yellow color of the polymer plate at the point of irradiation (Fig. 9b).

As a final proof-of-principle model IOLs having a 'Saturn-ring' haptic were fabricated. The IOLs were prepared from polymer plates by CNC diamond turning, polishing and extraction with physiological saline solution. Finally the IOLs were packaged and steam vapor sterilized. The diameter of the whole IOLs was 7.5 mm, the diameter of the lens was 5.0 mm having a thickness of 0.9 mm. The optical power was determined to be 22.5 dpt (Fig. 10a).

Finally the release of unmodified 5FU by TPA excitation at 532 nm in therapeutical relevant amounts of about 0.5–1.0 μg per dose from an UV-absorber containing foldable hydrophilic IOL was demonstrated. The drug diffused out into the aqueous environment within minutes. The IOL was irradiated two times with 0.65 kJ at 532 nm in 1.5 ml of physiological saline solution. The absorption at 268 nm in Fig. 10b corresponds to the released 5FU. After irradiation with 1.3 kJ the concentration of released 5FU reached 1.5 $\mu g\,ml^{-1}$, proving that the estimated therapeutical dosage of 1.0 $\mu g\,ml^{-1}$ was reached easily.

4. Summary and conclusion

We demonstrated that two-photon triggered drug release from polymers can easily be accomplished in the presence of an UV-absorber. The UV absorber should be selected to have a minimum of absorption at the wavelength used for excitation of the molecular moiety excited to uncage the drug. In this manuscript we used a new model compound with an *ortho*-nitrobenzyl-5-fluorouracil

functionality for single-photon and two-photon drug release. The quantum yield for SPA induced drug delivery could be calculated to 0.27 and the TPA cross section at 532 nm was found to be 2.41 GM. In presence of the BHP-EA no significant decrease of the TPA induced drug release in solution was measured.

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