

## Optimisation of two-photon induced cleavage of molecular linker systems for drug delivery

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### ABSTRACT

Photoreactive linker systems for drug delivery in modern ophthalmology are crucial for the efficiency of such applications. We compare 5 different linker molecule candidates (truxillic acid and 4 dimers of coumarin derivatives) in solution regarding their chemical stability and two-photon induced cleavage efficiency, and shed light into the role of molecular structure in the cleavage reaction. Dimers of the coumarin family showed much higher two-photon induced cleavage efficiency, achieving the highest reaction cross-section when *tert*-Butyldimethylsilyl (TBS) was used as a substituent, being almost 5-times higher than the unsubstituted coumarin dimer. Coumarin-based linker systems react promptly with nucleophilic solvents, in particular with short chain alcohols. The polarity of such solvents which do not cause a lactone ring opening seems not to influence the cleavage of the cyclobutane ring in coumarin dimers.

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### 1. Introduction

New drug delivery methods are a crucial aspect in several fields of modern medicine because they promise precise targeting of patients' diseases and optimised administration. Novel technologies based on gene therapy, cyclodextrins and lipid vesicles, just to mention a few, enabled new ways of overcoming drug absorption barriers in the skin and the mucosa [1]. Particularly in ophthalmology, a new photochemical drug delivery system has been recently suggested which is rapidly developing to become a valuable non-invasive therapeutic tool in the treatment of secondary cataract [2–4]. In this new device, the drug delivery is based on the photo-induced cleavage of a linker system which immobilizes the drug to an artificial drug-loaded polymeric intraocular lens (IOL).

An important aspect of this concept is the excitation mechanism of the linker system. In order to reach precise temporal targeting at the beginning of secondary cataract, the linker system must not be cleaved upon exposition to sunlight, which cannot be avoided in the eye. Two-photon excitation can be used with unchallengeable advantages: Multiphoton processes require high peak-intensities, which makes the drug release specific not just in time but also gives a high spatial resolution to the method [5]. When combined with linker systems that absorb in the UV spectral region, commercial

lasers with emission in the visible can be easily used as two-photon excitation sources [6].

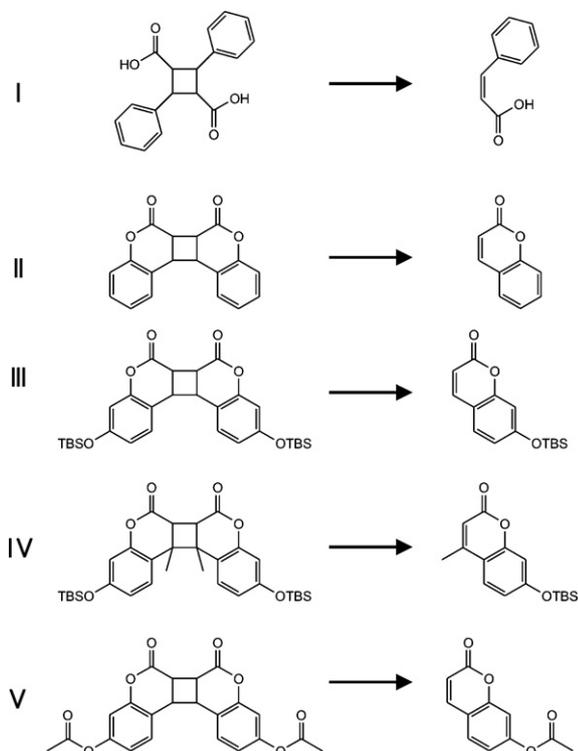
In order to achieve an effective treatment, i.e. a sufficient quantity of the drug released within a short irradiation time, an efficient linker system must be found. In previous works, we have explored coumarin derivatives as linker systems [2,7]. Coumarin dimers in solution show a two-photon absorption cross-section of around 1 GM and a reaction quantum yield of about 20–23%. In this contribution we investigate and compare systematically the cleavage efficiency of 5 linker systems (Fig. 1). Firstly, we compare two linkers based on different molecular families, truxillic acid (**I**) and unsubstituted coumarin dimer (**II**). Secondly, we investigate the effect of different substituents in 7-position of coumarin dimers on the total reaction cross-section. For this purpose we compare compounds **III** and **V** to the unsubstituted compound **II**. Thirdly, the effect of methylation of the cyclobutane ring in 4-position on the total reaction cross-section is investigated by comparing **III** and **IV**.

### 2. Experimental

#### 2.1. Sample preparation

All dimer molecules were synthesized as described in Ref. [2,8,9] and used after HPLC purification. Acetonitrile (Fluka, for UV-spectroscopy), ethanol (Sigma Aldrich, absolute), methanol (Fisher Scientific, HPLC grade) and isopropanol (Acros Organics, HPLC grade) were used without further purification. Acetonitrile

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**Fig. 1.** Structures of compounds I–V and respective monomers. (I) Truxillic acid, (II) coumarin dimer, (III) *tert*-Butyldimethylsilyl (TBS) – coumarin dimer, (IV) *tert*-Butyldimethylsilyl (TBS) –4-methyl-coumarin dimer, (V) acetoxycoumarin dimer.

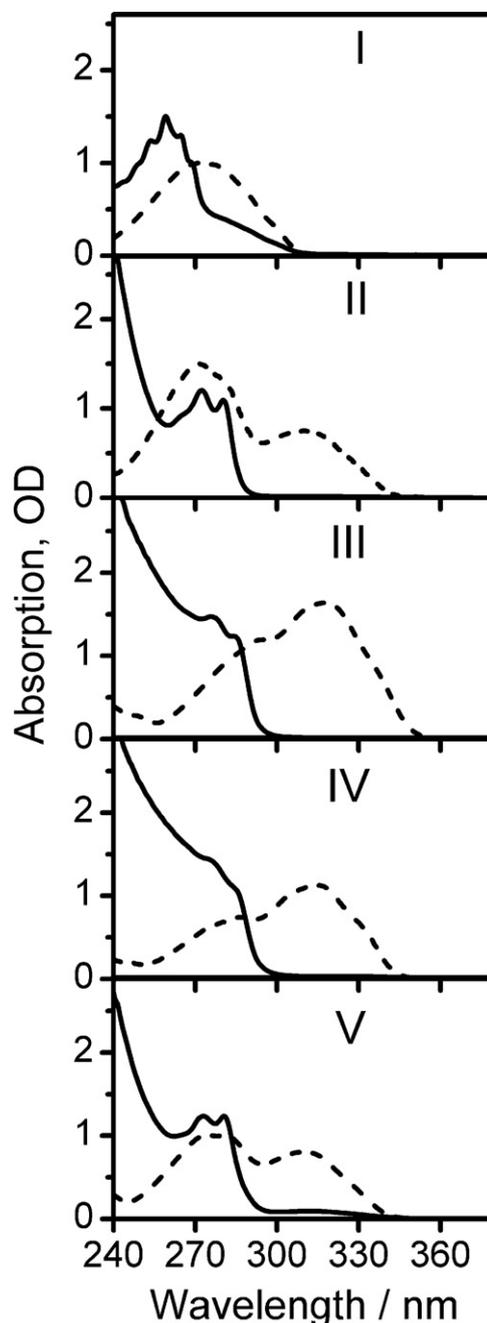
was selected as solvent and used in the irradiation studies for all linker systems (Section 2.3). Approx. 10 mg of each compound was dissolved in 10 mL acetonitrile yielding concentrations of about 2–3 mol/L. In order to calculate the concentration changes and the reaction cross-sections, the molar absorption coefficient was determined for all 5 compounds and is summarized in Table 1.

## 2.2. Irradiation setup

The irradiation study was performed using an Nd-YAG laser (Coherent, Infinity) with emission at 532 nm. Typical intensities between 3.6 and 6.5 W (at 100 Hz repetition frequency) with pulse durations of 3 ns were used for all linker systems. The pulse form was carefully checked for possible artefacts with a fast photodiode. The irradiation volume is defined by laser beam area and cell thickness. In our case it was typically 40  $\mu$ L, a factor 63 smaller than the typical sample volume. In order to maintain a homogenous concentration while the reaction proceeds, a magnetic stirrer was used

**Table 1**  
Molar absorption coefficients for compounds I–V in acetonitrile.

Compound	$\lambda$ (nm)	$\epsilon$ (L/mol cm)
Monomer		
I	272	19,965
II	310	5,170
III	317	13,845
IV	314	14,664
V	310	8,309
Dimer		
I	259	511
II	272	3,686
III	276	4,067
IV	276	4,456
V	260	3,557



**Fig. 2.** Linear absorption spectra of compounds I–V (continuous line) and respective monomers (traced line) measured in acetonitrile.

during the measurements. For the coumarin dimers (compounds II–V), the linear absorption change at the absorption maximum around 320 nm was used to follow the photo-induced cleavage, because dimer and monomer absorption are well separated and do not interfere with each other at this wavelength (Fig. 2). In case of compound I, the dimer and monomer absorption overlap one another. However, the absorption coefficient of the dimer is about a factor 1000-times smaller than that of the monomer, justifying the use of the absorption change at 272 nm in this case.

In order to improve the absorption measurement precision, the duration of irradiation was chosen so that the absorption change at the observed wavelength sums up to 0.20–0.30 OD. For example, for compound I the irradiation time was 4 h, while for compound V it was less than 1 h.

### 2.3. Chemical stability

The determination of the two-photon cleavage reaction efficiency requires accurate knowledge of laser parameters and sample concentrations before and after the photochemical reaction. In this context, an instable photoproduct or reactant may interfere with the calculation of reaction and two-photon cross-sections. Coumarin dimers tend to hydrolyze the lactone ring by nucleophilic attack as found previously for other derivatives [10]. For this reason methanol and other nucleophilic solvents must not be used in such measurements. Acetonitrile is a solvent for all investigated compounds and also no lactone ring cleavage has been observed. For these reasons acetonitrile was used throughout all the experiments as a solvent.

### 3. Results and discussion

The two-photon induced cleavage was followed by recording the absorption difference before and after irradiation. The quantification of the reaction and two-photon absorption cross-section was performed as in [11,12]. The reaction cross-section  $\Omega_{2PA}$  is given by the product of the two-photon absorption cross-section  $\sigma_{2PA}$  and the reaction quantum yield  $\phi_{2PA}$ :

$$\Omega_{2PA} = \sigma_{2PA} \times \phi_{2PA}. \quad (1)$$

The quantum yield was obtained from one photon irradiation for compound **III** and, due to almost constant values for previously investigated coumarin dimers, was also assumed to be constant here. This assumption is based on the experimental observation that the linear absorption spectrum scales perfectly with the wavelength dependence of the two-photon induced reaction [7]. Similar assumption was made for the quantum yield of truxillic acid [13]. The results are summarized in Table 2.

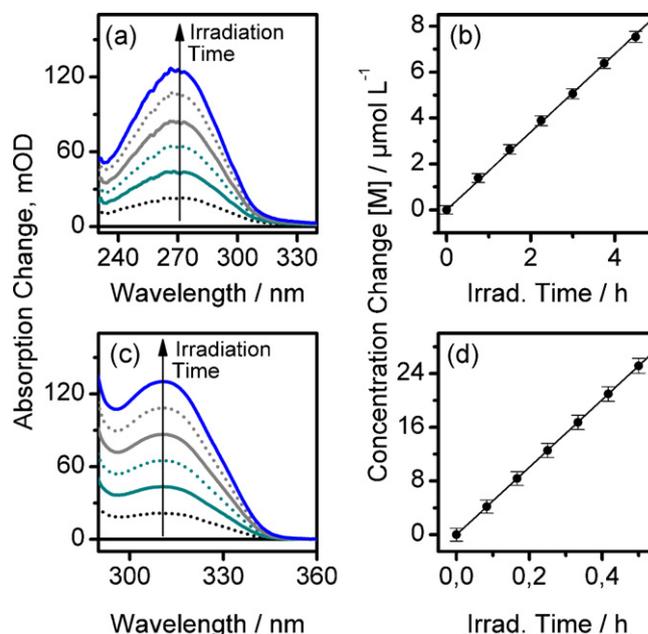
For compounds **I** and **II**, the absorption change upon irradiation is exemplarily shown in Fig. 3. The two-photon cleavage efficiency of the coumarin dimer exceeds that of truxillic acid (**I**) by a factor 30. With similar experimental irradiation parameters, compound **I** requires almost 5 h of irradiation to achieve just one third of the turnover achieved with compound **II** in 30 min of irradiation. This strong difference must be caused by the two-photon absorption cross-section, since the quantum efficiency for the coumarin dimer photocleavage is a factor 2 better than for the truxillic acid [13]. This means that the  $\sigma_{TPA}$  for truxillic acid is still a factor 11 worse than for compound **II**.

Compounds **III** and **IV** share the same substituent in 7-position but differ by the presence of two methyl groups on the cyclobutane ring. In the past we speculated that due to deformation in the ring caused by the methyl substituents, compounds with this feature should be cleaved easily and thus allow a more efficient overall photo-induced reaction. They did not fulfil this expectation, but both compounds showed within our precision limits similar reaction cross-sections. The most important molecular feature for the reaction efficiency is the substituent in 7-position. All substituted coumarin compounds (**III–V**) show a higher reaction cross-section

**Table 2**

The pulse energy  $E_{\text{pulse}}$ , dimer initial concentration  $D_0$ , monomer concentration variation ( $\Delta C^{\text{mono}}$ ) with the respective irradiation time, cleavage cross-section ( $\Omega_{2PA}$ ), photo-induced cleavage yield ( $\phi_{2PA}$ ) and absorption cross-section ( $\sigma_{2PA}$ ) for two-photon induced reaction [1 GM (Göppert Meyer) =  $10^{-50}$  cm<sup>4</sup> s photon<sup>-1</sup>].

Compound	$E_{\text{pulse}}$ (mJ)	$D_0$ ( $\mu\text{M}$ )	$\Delta C^{\text{mono}}$ ( $\mu\text{M}$ )	$\Omega_{2PA}$ (GM)	$\phi_{2PA}$ (%)	$\sigma_{2PA}$ (GM)
<b>I</b>	65	2.0	8 [4.5 h]	$2.69 \times 10^{-3}$	12.3	$0.02 \pm 0.01$
<b>II</b>	66	2.5	25 [30 min]	$0.05 \pm 0.1$	23.1	$0.22 \pm 0.02$
<b>III</b> (in MeCN)	36	1.6	16 [30 min]	$0.23 \pm 0.03$	23.1	$1.01 \pm 0.02$
<b>III</b> (in iPrOH)	37	1.6	18 [30 min]	$0.24 \pm 0.01$	23.1	$1.02 \pm 0.03$
<b>IV</b>	35	1.7	15 [30 min]	$0.22 \pm 0.03$	23.1	$0.97 \pm 0.11$
<b>V</b>	36	3.7	24 [45 min]	$0.09 \pm 0.01$	23.1	$0.37 \pm 0.01$

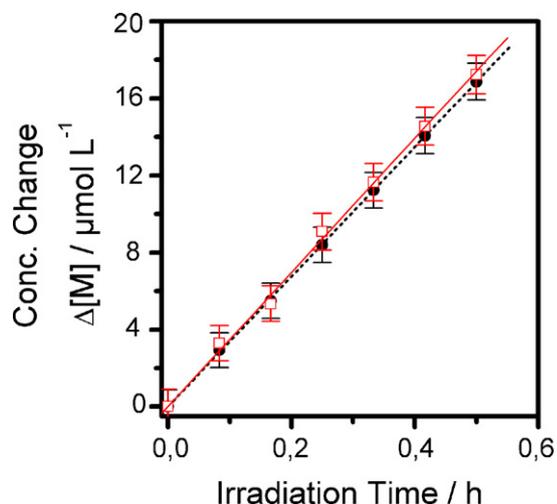


**Fig. 3.** Absorption change for compounds (a) **I** and (c) **II**. The evolution of the absorption change with the irradiation time is shown in (b) for compound **I** and in (d) for compound **II**.

$\Omega_{2PA}$  than the unsubstituted coumarin compound **II**. In particular compounds **III–IV**, which share the same substituent, exhibit a reaction cross-section about 5-times higher than compound **II**.

For non-centrosymmetric molecules in the two-states approximation, the TPA depends on the strength of transition dipole moment and the dipole moment changes between the excited and ground state [14,15]. Coumarins are molecules with a first excited state  $S_1$  showing a strong intramolecular charge transfer (ICT) character, which implies a much larger dipole moment than in the  $S_0$  [16]. The symmetric arrangement of the carboxyl and phenyl groups in truxillic acid suggests a higher quadrupole than a dipole character. The transition dipole moment of this dimer is so small that single-photon absorption is negligible compared to cinnamic acid (monomer of compound **I**) (see Fig. 1). Although it is known for  $\pi$ -conjugated systems that chain length extension results in an increase of the transition dipole moment, the effect of dimerization on the ICT character for the  $S_1$ -state of the coumarin monomer is unknown at present. Despite of that, the ICT property of the  $S_1$ -state of coumarins together with the sensible strong quadrupolar moment of truxillic acid may be an explanation for the much bigger TPA cross-section of coumarin dimers compared to truxillic acid.

In  $\pi$ -conjugated systems, the TPA cross-section can usually be optimised using electron donor and acceptor groups. TPA of coumarin dimers can be enhanced in a similar way. For example, the carbonyl groups in compounds **II–V** act as electron donor while substituents with different electron donor/acceptor qualities can be added. The variation of the reaction cross-section observed



**Fig. 4.** Investigation of the cleavage reaction efficiency of compound **III** for two solvents (a) acetonitrile (open squares with continuous line) and isopropanol (filled circles with traced line).

for compounds **II–V** is compatible to complementary experimental observations [17] and theoretical calculations [16,18] for coumarin monomers. In Ref. [16], the transition dipole moment was shown to scale with the degree of alkylation of aminocoumarin derivatives. The TPA cross-section increased upon increasing the number of alkyl groups in Coumarin 120, Coumarin 1 and Coumarin 480. Even for trifluorinated coumarins, the calculated TPA follows the same alkylation trends found for the aminocoumarins. In case of the compounds investigated in this work, compound **I** does not have any alkyl substituents and therefore showed the smallest reaction efficiency. Compounds **III** and **IV**, which share very similar structures and a higher degree of alkylation than the other compounds, showed the highest reaction efficiency. Compounds **III** and **IV** have three times more alkyl groups than compound **V** but the increase of the TPA cross-section was smaller (about a factor 2–3). From this trend, it seems that the TPA cross-section scales linearly with the number of alkyl groups, suggesting that alkylation is also an important control knob in the two-photon induced cleavage reaction even if the alkylation originates from a *tert*-Butyldimethylsilyl (TBS) group. It is important to note that two-photon absorption cross-sections may be slightly modified when measured with nanosecond pulses [19]. Such pulses can re-pump excited state populations and thereby achieve higher reaction turnovers, as we observed in a previous work [7].

Apart from the inherent amplitude of the molecular TPA cross-section, investigating the effect of the environment on the photocleavage efficiency would be interesting. The polarity of the environment is an important aspect in the photoreaction evolution because generally polar environments tend to lower the reaction activation energy. We investigated the reaction efficiency of compound **III** in two solvents with different polarity and proticity, acetonitrile ( $\epsilon = 37$ ) and isopropanol ( $\epsilon = 18$ ). The investigation of chemical stability has shown that compound **III** is equally stable in both solvents. The lack of any solvent dependence suggests that the reaction is barrierless or that intermediate species are not well stabilized by the solvent. This is interesting from the practical point of view, since it implies that reaction may evolve similarly in the future medical application in environments with high water content. Furthermore, during the irradiation time of 30 min, degradation due to solvent-induced lactone ring opening can be neglected and a direct comparison of the reaction turnover is possible without any correction (see Table 1). Fig. 4 shows that the two-photon induced cleavage of compound **III** is similar for both solvents.

This result is an additional information about the reaction mechanism and the chemical stability of the coumarin dimer in polar environments. Hasegawa and co-workers investigated the photochemical behaviour of several dimers of coumarin derivatives, focusing on the cleavage reaction pathway after single photon excitation [10]. Depending on the coumarin derivative, nucleophilic solvents like alcohols lead to asymmetric fission of the cyclobutane ring, giving two different species (not the monomer). Thus, since in our case the reaction cross-section for both solvents was the same, we can safely affirm that cleavage of compound **III**, the compound with the most effective reaction, evolves over a symmetric reaction pathway in these two solvents as confirmed by HPLC analysis. This is of importance in technical applications if the reverse reaction, i.e. dimerization, is to be exploited as, for example, in optical lenses with tuneable refractive properties based on these systems [20].

#### 4. Conclusions

The investigation of photo reactive linker systems for drug delivery in ophthalmology is a crucial step in the optimisation of this new technology. Linker systems must be chemically stable and present a high reaction cross-section for efficient implementation. In this work we have investigated several linker systems for potential use in a drug delivery method dedicated to ophthalmology. Using a two-photon induced process, we systematically characterized the two-photon reaction cross-section of 5 different systems. Dimers from the coumarin family showed the highest photo-induced cleavage efficiencies. Furthermore, we discovered that substituents modify strongly the reaction cross-section, improving the photoreaction almost by a factor 5. Complementing previous results for the dimerization of coumarin molecules in solution, the dimer cleavage seems to evolve independent of the environment when non-nucleophilic solvents are used in order to assure chemical stability.

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#### References

- [1] B. Wang, T. Sahaan, R. Soltero (Eds.), *Drug Delivery: Principles and Applications*, John Wiley & Sons, Inc, Hoboken, 2005.
- [2] H.-C. Kim, S. Kreiling, A. Greiner, N. Hampp, Two-photon-induced cycloreversion reaction of coumarin photodimers, *Chemical Physics Letters* 372 (2003) 899–903.
- [3] H.-C. Kim, S. Haertner, M. Behe, T.M. Behr, N.A. Hampp, Two-photon absorption-controlled multidose drug release: a novel approach for secondary cataract treatment, *Journal of Biomedical Optics* 11 (2006), art. no. 034024.
- [4] J. Trager, H.C. Kim, N. Hampp, Ophthalmology—two-photon treatment, *Nature Photonics* 1 (2007) 509–511.
- [5] K. König, Multiphoton microscopy in life sciences, *Journal of Microscopy: Oxford* 200 (2000) 83–104.
- [6] H.C. Kim, S. Hartner, N. Hampp, Single- and two-photon absorption induced photocleavage of dimeric coumarin linkers: therapeutic versus passive photocleavage in ophthalmologic applications, *Journal of Photochemistry and Photobiology A: Chemistry* 197 (2008) 239–244.
- [7] T. Buckup, J. Dorn, J. Hauer, S. Hartner, N. Hampp, M. Motzkus, The photoinduced cleavage of coumarin dimers studied with femtosecond and nanosecond two-photon excitation, *Chemical Physics Letters* 439 (2007) 308–312.
- [8] S. Hartner, H.C. Kim, N. Hampp, Photodimerized 7-hydroxycoumarin with improved solubility in PMMA: single-photon and two-photon-induced photocleavage in solution and PMMA films, *Journal of Photochemistry and Photobiology A: Chemistry* 187 (2007) 242–246.
- [9] Y. Chen, R.-T. Hong, Photopolymerization of 7,7'-coumarinyl polymethylene dicarboxylates: fluorescence and kinetic study, *Journal of Polymer Science, Part A: Polymer Chemistry* 35 (1997) 2999–3008.
- [10] N. Yonezawa, T. Yoshida, T. Hasegawa, Symmetric and asymmetric photocleavage of the cyclobutane rings in head-to-head coumarin dimers and their lactone-opened derivatives, *Perkin Transactions 1* (1983) 1083–1086.
- [11] D.M. Friedrich, Two-photon molecular spectroscopy, *Journal of Chemical Education* 58 (1982) 472–481.

- [12] N.K. Urdabayev, A. Poloukhine, V.V. Popik, Two-photon induced photodecarbonylation reaction of cyclopropanones, *Chemical Communications* (2006) 454–456.
- [13] J. Rennert, D. Grossman, Photochemical scission of cinnamic acid dimers: alpha-truxillic and beta-truxinic acids, *Journal of Photochemistry* 3 (1974) 163–170.
- [14] P.N. Day, K.A. Nguyen, R. Pachter, Calculation of two-photon absorption spectra of donor-pi-acceptor compounds in solution using quadratic response time-dependent density functional theory, *Journal of Chemical Physics* 125 (2006) 094103.
- [15] B. Strehmel, V. Strehmel, in: D.C. Neckers, W.S. Jenks, T. Wolff (Eds.), *Advances in Photochemistry*, John Wiley & Sons, Hoboken, 2008, pp. 111–354.
- [16] K.A. Nguyen, P.N. Day, R. Pachtera, Effects of solvation on one- and two-photon spectra of coumarin derivatives: a time-dependent density functional theory study, *Journal of Chemical Physics* 126 (2007) 094303.
- [17] A. Samanta, R.W. Fessenden, Excited-state dipole moment of 7-aminocoumarins as determined from time-resolved microwave dielectric absorption measurements, *Journal of Physical Chemistry A* 104 (2000) 8577–8582.
- [18] R.J. Cave, K. Burke, E.W. Castner, Theoretical investigation of the ground and excited states of Coumarin 151 and Coumarin 120, *Journal of Physical Chemistry A* 106 (2002) 9294–9305.
- [19] R.L. Sutherland, M.C. Brant, J. Heinrichs, J.E. Rogers, J.E. Slagle, D.G. McLean, P.A. Fleitz, Excited-state characterization and effective three-photon absorption model of two-photon-induced excited-state absorption in organic push-pull charge-transfer chromophores, *Journal of the Optical Society of America B: Optical Physics* 22 (2005) 1939–1948.
- [20] J. Trager, J. Heinzer, H.C. Kim, N. Hampp, Polymers for in vivo tuning of refractive properties in intraocular lenses, *Macromolecular Bioscience* 8 (2008) 177–183.