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Chemicals on demand:

Photo-induced release from nano- and microparticle containers

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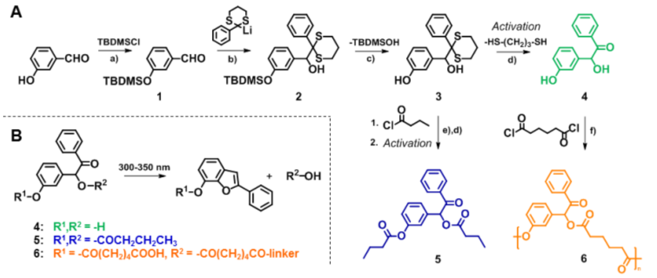
Abstract: A benzoin-derived diol linker was synthesized and used to create biocompatible polyesters, which can be fully decomposed on demand triggered by UV light. Extensive structural optimization of the linker unit was performed to enable a defined encapsulation of structurally diverse organic compounds in polymeric structures and a well-controllable polymer cleavage process. Selective tracking of release kinetics of encapsulated model compounds from the polymeric nano- and microparticle containers was enabled by confocal laser scanning microscopy as a proof-of-principle for desired applications. The model compounds comprised physico-chemical properties from fully hydrophilic to fully hydrophobic. Demonstrated biocompatibility of the utilized polyesters and degradation products enables advanced applications, e.g. for smart packaging of UV-sensitive pharmaceuticals or nutrition components, or even in the area of spatially-selective self-healing processes.

The incorporation of stimuli-responsive units into polymer networks imparts smart properties to these materials, thus enabling a controlled release of compounds by external triggers.[1] Such concepts can be realized by different approaches. One of them is the use of cleavable, bifunctional linker units, which represents a method for triggering the complete decomposition of the polymeric material using external stimuli.[2] Among the different stimuli, light is of outstanding importance. This is because it can be applied in a very precise manner by selecting suitable wavelengths, polarization directions, and intensities in an essentially non-contact approach. Numerous photo-cleavable polymers, such as polyurethanes,[3] polyacrylates[4], polyesters[5] or polyethers,[6] have been reported. Nitroaryl groups, i.e. *o*-nitrobenzyl groups, are by far the most commonly used photo-responsive linker units.[7-9] Concerning particular applications, they suffer from serious drawbacks. Apart from slow degradation kinetics and unwanted by-products of the photolysis reaction by the use of *o*-nitrobenzyl linkers, these are recognized as reactive chromogenic nitroso carbonyl compounds, revealing severe cytotoxicity.[10-11] Even in derivatives, where cleavage kinetics have been improved to as much as 95% in 3 h,[12] issues such as lengthy synthesis, sensitivity to sunlight, and reactive side products remain challenging. This limits their applicability for advanced life science settings. Alternative methods comprise photo-responsive phenacyl, coumarin-4-ylmethyl, or arylmethyl groups. Most common photo-degradable compounds are monofunctional, rendering them unsuitable to be part of a polymer backbone. The use of a dithiane protected benzoin derivative (from the group of arylcarbonylmethyl), firstly described by Sheehan *et al.* as a light-sensitive protecting group for carboxylic acids,[13] may circumvent such limitations. Established for peptide[14] and solid-phase synthesis,[15-16] the use of diol benzoin derivatives as an active monomer in the polymerization process of polyesters has, surprisingly, to the best of our knowledge, not been reported so far. In this study, we focus on the synthesis of nano- and microparticle containers, which can be "opened" by UV light in a simple and well-controlled manner while releasing chemicals on demand within defined periods of time (within timescales of minutes to hours). In order to enable potential biomedical, environmental, and ecological applications, the particles are based on a biocompatible polyester.



Scheme 1. Schematic representation of A) the synthesis of the photo-sensitive linker 4 (green) and 5 (blue) as well as polyester 6 (orange), and B) their cleavage after UV irradiation for defined time scales. a) *tert*-butyl(chloro)dimethylsilane (TBDMSCl), THF, triethylamine, 0 to 20 °C; b) 2-phenyl-1,3-dithiane, n butyllithium, THF, 0 °C; c) Cs_2CO_3, DMF/H_2O; d) mercury(II) perchlorate hydrate, CH_3CN/H_2O; e) 4 dimethylaminopyridin, butyryl chloride, CH_2Cl_2; f) adipoyl dichloride, THF, pyridine.

For this purpose, a dithiane protected diol was synthesized and activated as well as used for the step growth polymerization in order to incorporate it into the polyester structure. The inactive

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diol precursor 3-(hydroxy(2-phenyl-1,3-dithian-2-yl)methyl)-phenol (Scheme 1A, **3**) was synthesized in a three-step approach *via* a Corey-Seebach addition reaction starting from 3-hydroxybenzaldehyde.[14] Subsequently, the product was activated to obtain 2-hydroxy-2-(3-hydroxyphenyl)-1-phenyl-ethan-1-one (**4**), or esterified using butyryl chloride prior to activation (**5**). The experimental data as well as the spectroscopic and spectrometric characterization can be found in the Supporting Information (ESI). The NMR evaluation, including ^{1}H NMR-, ^{13}C NMR-, HSCQ-, NOESY- and ROESY-spectroscopy, is exemplified for the esterified linker **5** (see ESI, Figures S10-S13). The use of crystallization instead of column chromatography for purification renders the synthesis of the photo-degradable linker very efficient compared to other approaches. The cleavage mechanism of benzoin derived linker structures is depicted in Scheme 1B. Upon UV irradiation (λ = 300 to 350 nm), the substituted benzoin undergoes a photosolvolytic cleavage process and subsequent rearrangement to an isomeric benzofuran derivative.[10] The dithiane protecting group prevents premature photolysis, which increases the shelf-life of the linker-precursor (**3**).[14] The small linkers (**4**, **5**) were used for kinetic investigations and the development of a well-controlled release strategy under UV irradiation (UV cube with broadband radiation spectrum, see ESI, Figure S1). Figure 1A represents the proton NMR spectra of the esterified linker **5** before and after UV irradiation. A clear upfield shift of the phenyl Ph-2 protons at δ_H 7.96 ppm (signal K to K') indicates a successful degradation and was explored to determine the degree of cleavage. The disappearance of δ_H 6.87 ppm (signal D, -C*H*OR-) and the appearance of δ_H 7.05 ppm (signal X, newly formed aromatic proton) are further indices for the proposed cleavage mechanism. UV-VIS spectroscopy represents a simple and highly-sensitive method for further investigations concerning cleavage and stability of the photo-degradable linkers. The degradation kinetics for irradiation of linkers **4** and **5** are presented in Figure 1B. The absorption spectra of the cleaved linkers reveal an increasing absorption in the range of 275 to 325 nm (exemplified for linker **5** in Figure 1C).

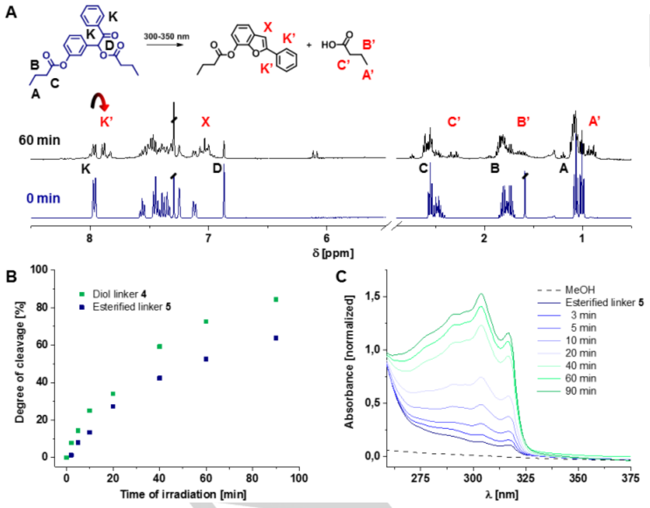

Figure 1. A) Schematic representation of the ^{1}H NMR spectra of the esterified linker 5 before (blue) and after UV irradiation (60 min, black) (300 MHz, $CDCl_3$); B) ^{1}H NMR kinetics of the cleavage of linker 4 (green) and esterified linker 5 (blue); C) UV-VIS absorption spectra monitoring the cleavage of the esterified linker **5**.

In order to form photo-degradable materials, the activated diol functional linker **4** was polymerized with adipoyl dichloride to obtain polyester **6** by polycondensation (see Scheme 1). Size exclusion chromatography could be used to determine the apparent weight-average molar mass (M_w) of 2,800 g mol^{-1} (SEC: $CHCl_3$/*i*PrOH/NEt_3, polystyrene standard). The step-growth polymerization results in several molar mass fractions. Under UV illumination, the population of the low molar mass fractions increases with irradiation time, while high molar mass species successively disappear (Figure 2A). While NMR characterization is possible for the polymeric species **6** (see ESI, Figure S15), the determination of the degree of cleavage is hindered by indistinct signals of the degradation products. UV absorption investigations showed similar cleavage kinetics for polymer **6** (see ESI, Figure S16) as obtained for the small molecule linkers **4** and **5**. A comparison of the UV absorbance after different times of irradiation at λ = 304 nm revealed a significantly faster degradation of the small molecule linker **5** compared to the polymeric species (Figure 2B). This fact can be explained with the preferred leaving group character of butyric acid. Further UV absorption measurements of the polyester confirmed stability at increased temperatures (up to 80 °C) and, as expected, decomposition under daylight, which occurs at significantly slower rate compared to direct UV irradiation (see ESI, Figure S17).

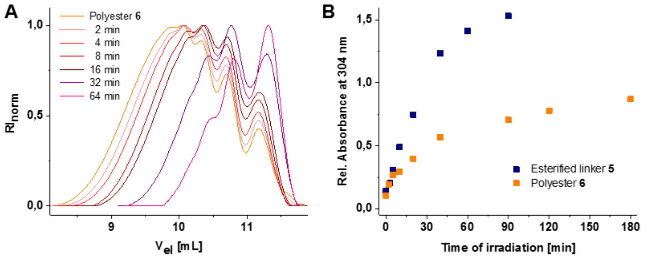

Figure 2. A) Size exclusion chromatography of the photo-degradable polyester **6** treated with UV light for different time scales (*N*,*N*-dimethylacetamide, 0.21% LiCl); B) UV absorption to monitor the kinetics of the cleavage of the photo-sensitive linker **5** and the polyester **6** at the defined absorption wavelength of λ = 304 nm.

To allow a well-controlled release of chemicals on demand, the formation of microparticle containers from the presented polymers was approached. Using the (double-) emulsion technique,[17] a hydrophobic (Nile Red) as well as a hydrophilic dye (Rhodamine B) were incorporated in microparticle containers composed of the photo-cleavable polyester (Nile Red: **7n**, Rhodamine: **7r**). Scanning electron microscopy confirmed particle sizes in a range of 2 to 7 µm (Figure 3A). This microparticle approach allows a fast and simple production of containers cleavable under UV irradiation, with the dyes being stable under such conditions (see ESI, Figure S18). The cargo revealed different behavior upon UV triggered degradation of the microcontainers, in fact due to their varying solubility in the particle surrounding liquid phase. While Nile Red shows a clear decrease of the fluorescence intensity (due to precipitation of the dye in the hydrophilic environment outside the dispersed particles[18]), Rhodamine B release results in increased fluorescence intensities outside the microcontainers (Figure 3B). To illustrate that even short UV irradiation times are sufficient to generate a significant release of cargo from such particles, the microcontainers were exposed to UV light for 10 s and then kept in the dark to monitor such imposed release characteristics. The experiment revealed a direct release of ca. 20% of the cargo (i.e. Nile Red) followed by a kinetically slow release over a timescale of 16 min (20%, Figure 3B). This behavior could be used in future applications for smart packaging of UV-sensitive

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pharmaceuticals or nutrition components. The biocompatible containers can be loaded with dyes of different physicochemical properties, demonstrated here for the two example cases of a fully hydrophobic and a fully hydrophilic dye. In addition, dye-loaded containers could be added in pharmaceutical formulations, *e.g.*, for spatially ensuring the absence of harmful irradiation.

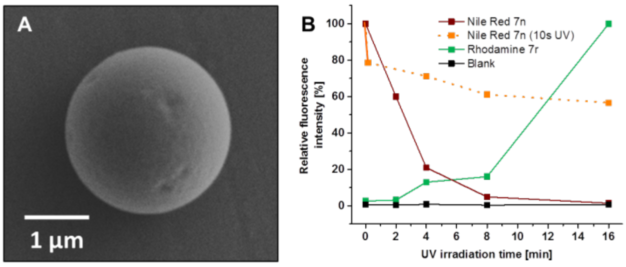

Figure 3. A) Scanning electron microscopy image of Nile Red encapsulated microparticle **7n**; B) cleavage of dye encapsulated microparticles under UV irradiation (Nile Red: **7n**, red & orange; Rhodamine B: **7r**, green; blank: black).

The wavelength-dependent photo-induced release from the microparticle containers could be visualized by confocal laser scanning microscopy. As a proof-of concept, Nile Red encapsulated microparticles were irradiated *via* a laser, emitting light at $\lambda = 405$ nm. Since this wavelength is outside the absorption band of the polyester, no change in fluorescence intensity could be observed after 10 cycles (1 cycle = 1 s laser irradiation, 10% laser intensity) (Figure 4A). In contrast, laser irradiation at $\lambda = 355$ nm in the same timescale resulted in a clear decrease of the fluorescence intensity within the exposed area caused by a release of the cargo (Figure 4B). An immediate zoom-out of the irradiated region after 10 cycles further illustrates the local precision of the photo-induced cleavage process (Figure 4C). Thus, we demonstrated that defined irradiation in the submillimeter range ensures selective release at the desired location.

Concerning potential life science applications, such as biomedical and ecological, biocompatibility represents a very critical parameter. *In vitro* studies with L929 cells confirmed no cytotoxicity of the polyester-based microparticles and their respective cleavage products after 2 and 16 min UV irradiation with IC_{50} values > 500 µg mL^{-1} (see ESI, Figure S19). Furthermore, it was demonstrated that the containers can also be scaled-down successfully to nanoparticulate containers using the emulsion technique, yielding photo-degradable nanometer-scale light-responsive objects (see ESI, Table S1, Figure S20). *In vitro* studies confirmed no cytotoxicity of the nanoparticulate containers and their degraded fragments even when approaching the highest degrees of cleavage, consequently degradation, according to Figures 1 and 2. After 60 and 120 min UV irradiation, IC_{50} values > 300 µg mL^{-1} were detected (see ESI, Figure S21).

In summary, we present a new approach for the efficient photo-induced degradation of nano- and microparticle containers to release chemicals on demand with different physicochemical properties, opening the gate for numerous applications. With a bottom-up design, starting from the synthesis of a bifunctional photo-sensitive benzoin linker, we were able to create polyester structures that can be fully decomposed on demand, triggered by UV light. The formulation and design of biocompatible nano- and microparticle containers by emulsion techniques allowed the encapsulation and targeted release of organic compounds at the desired location of interest, evidenced by confocal laser scanning microscopy. The controlled “opening” of the containers can be performed by a single dose of UV light, followed by retained and relatively slow release or further accelerated release by prolonged UV light exposure. The concept can be widened by using different tailored polymer classes for drug delivery applications, *e.g.* for the controlled release of particular drugs of interest *via* endoscopy, smart packaging of UV-sensitive pharmaceutical or nutrition components, or even in the area of spatially-selective self-healing processes.

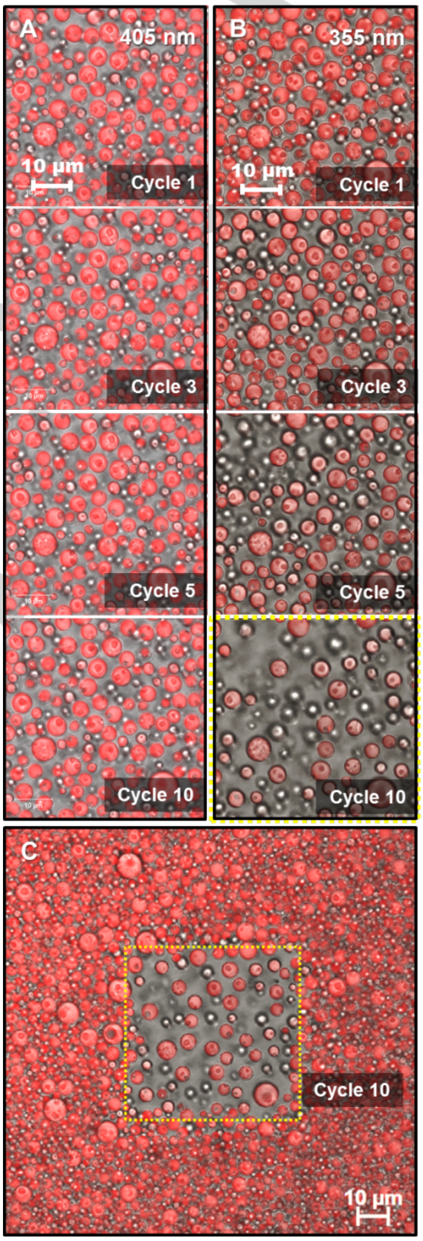

Figure 4. Confocal laser scanning microscopy (CLSM) of photo-cleavable polyester-based microparticles with encapsulated Nile Red **7n** (1 cycle = 1 s laser irradiation, 10% laser intensity): A) laser irradiation at 405 nm; B) laser irradiation at 355 nm; C) immediate zoom-out of the irradiated region after 10 cycles.

Experimental Section

Full experimental details are provided in the Supporting Information.

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Keywords: micro- & nanostructures • photochemistry • polyesters • benzoin-derivative linker • UV light

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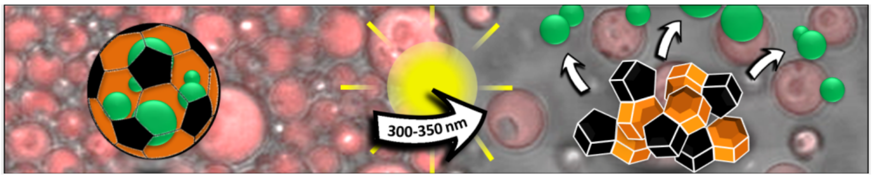

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