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Communication

Fluorescent Polymers from Non-Fluorescent Photoreactive Monomers

Jan O. Mueller,[a,b] Dominik Voll,[a,b] Friedrich G. Schmidt,[c] Guillaume Delaittre*[a,d] and Christopher Barner-Kowollik*[a,b]

**A facile, fast and ambient-temperature avenue towards highly fluorescent polymers is introduced *via* polymerizing non-fluorescent photoreactive monomers based on light-induced NITEC chemistry, providing a platform technology for**
10 **fluorescent polymers. The resulting polypyrazolines were analyzed in depth and the photo-triggered step-growth process was monitored in a detailed kinetic study.**

During the last decades the importance of photo-induced processes has rapidly increased not only in organic or
15 biochemistry, yet also in materials science and polymer chemistry. The need for efficient light-triggered ligation techniques is particularly present in the field of polymer science, where post-polymerization transformations and purification procedures can be extremely challenging. Due to their appealing
20 properties (e.g. high yields, facile conduction, limited – if any – side reactions, spatiotemporal control), a variety of photo-induced ligation techniques has been established in polymer-focused applications including (hetero) Diels–Alder systems (photoenol[1] and *o*-naphthoquinone[2]), [2+2]-cycloadditions,[3] thioaldehyde
25 based processes,[4] and [3+2]-cycloaddition systems.[5] Among the later reactions, the most widely employed system is the nitrile imine-mediated tetrazole-ene cycloaddition (NITEC), which has been used in numerous examples for e.g., protein-polymer conjugation reactions,[6] spatially resolved surface modification on
30 various substrates,[7] and the generation of fluorescent single-chain nano particles.[8] Especially the biocompatibility of the NITEC process, during which a nitrogen molecule is released and a pyrazoline species is formed, widens the scope of this methodology allowing for instance in-vivo protein
35 modifications.[9] Herein, we provide a novel application for light-triggered ligation techniques, presenting a polymerization strategy based on NITEC chemistry.

The NITEC concept enables addressing another highly topical field of research by generating a cycloadduct with fluorescent
40 properties.[10] Fluorescent polymeric materials have recently been employed for imaging applications,[11] bio-sensing,[12] fluorescence resonance energy transfer (FRET) analysis,[13] and polymeric light-emitting diodes.[14] They typically either consist of conjugated polymer backbones[15] or fluorescence markers which are attached
45 to non-fluorescent polymer backbones.[16] In our current approach, the fluorescent species is incorporated into the polymer backbone forming the connection between the monomer units in a step-growth polymerization process.

The concept of step-growth polymerization – first investigated
50 by Carothers[17] – has recently been revived by introducing novel efficient conjugation tools referred to as *click* chemistry.[18] A popular example is the copper-catalyzed azide alkyne cycloaddition, which was applied for instance to produce conjugated polymers,[19] poly(ferrocene)s,[20] or palladium
55 containing polymers.[21] To the best of our knowledge, there are only few examples where light-triggered cycloaddition reactions have been applied in the context of step-growth polymerization. Although polyimides were generated by polymerization of bis-maleimides with photoenol dilinkers[22] or benzene[23] and the
60 dimerization reactions of coumarin or cinnamate groups were employed for polymerizing oligoethyleneglycol monomers,[24] the NITEC approach has not yet been employed and neither of the former examples produces a fluorescent product.
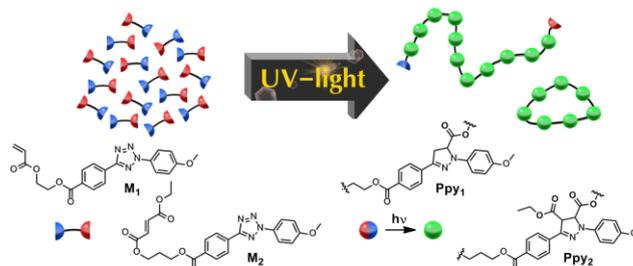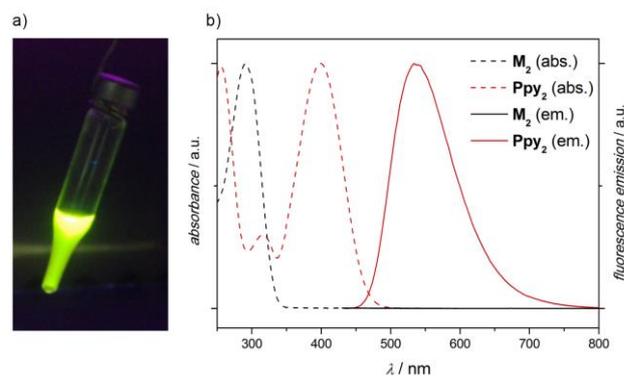

Scheme 1 General reaction scheme for the photo-induced polyaddition of
65 **M₁** and **M₂** forming **Ppy₁** and **Ppy₂** respectively.

Firstly, new photoreactive monomers had to be designed to enable a step-growth polymerization process (Scheme 1). In order to avoid the common problems with inaccurate molarities of the two reactive groups – typical for "A-A + B-B"-type of
70 polymerizations – our novel monomers were designed to be of the type A-B, expressing both functional species combined in one molecule. The synthetic pathway is simple (Scheme S1, Supporting Information): A carboxylic acid-functionalized diaryl tetrazole is transformed into the corresponding acyl chloride and
75 conjugated with a hydroxy terminal spacer molecule attached to the activated double bond species, either an acrylate (**M₁**) or a fumerate (**M₂**)(see Scheme 1). The molar masses of the resulting monomers are 394.4 g mol^{-1} (**M₁**) and 480.5 g mol^{-1} (**M₂**). Subsequent to the monomer preparation, the reaction conditions
80 for the novel polycycloaddition had to be explored since a variety of parameters were expected to influence the polymerization product: concentration, total batch size, irradiation time, and
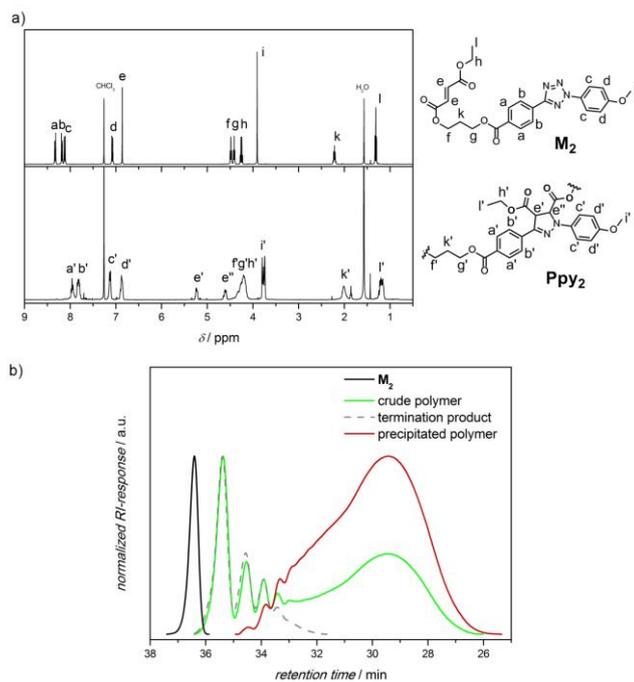
Fig. 1 a) NMR spectra of monomer **M₂** (top) and the crude polypyrazoline **Ppy₂** (bottom). b) SEC chromatograms of monomer **M₂** (black), its crude polymerization product (green), its non-precipitating termination product (dashed gray), and its precipitated polymer fraction (red). Polymerization conditions: **M₂** (2 mg) was dissolved in THF (100 mg mL⁻¹) and irradiated for 8 h (108 W).

Fig. 2 a) Image of a vial containing fluorescent **Ppy₂** solution excited under UV-irradiation (365 nm) of a hand-held TLC lamp. b) UV-Vis (dashed) and fluorescence (plain) spectra of **M₂** (black) and poly(pyrazoline) (**Ppy₂**, red).

irradiation intensity. Therefore, the polymerization reactions were conducted in a custom built photo-reactor, which can be equipped with 1, 3, or 5 lamps (for lamp specification and schematic picture of the photoreactor, refer to Fig. S10-S11). Upon UV light exposure, the excited tetrazole moieties each release a nitrogen molecule, forming a highly reactive nitrile imine species, which subsequently reacts with the dipolarophile units present in an equimolar amount in a [3+2]-cycloaddition. This cycloadduct can potentially either connect two monomers, oligomers, or polymeric species in a step-growth polymerization process or in an intramolecular α,ω-cycloaddition generating cyclic species and terminating the polymerization (Scheme 1).

In order to explore this novel photopolymerization concept, the reaction conditions had to be established. **M₁** was employed for the initial light-triggered reactions in order to determine the influence of the monomer concentration and of the total amount of monomer per batch (Fig. S1 and S2). Since the solubility of **Ppy₁** is reduced in organic solvents suitable for analytic methods, in-depth characterization was conducted with **Ppy₂** and the kinetic study refers to the polymerization process of **M₂**. The concentration was chosen to be as high as possible since the probability for intermolecular reaction between two different polymer chains is less likely in diluted systems, where intramolecular cycle formation is favored. This assumption was verified in an initial concentration study with **M₁**, depicted in Fig. S1. Consequently, a concentration of 100 mg mL⁻¹ was employed for all further polymerization reactions. In addition, the total batch size needed to be established. Since the reaction time to achieve full conversion scales with the total amount of monomer utilized (Fig. S2), the minimum amount required for
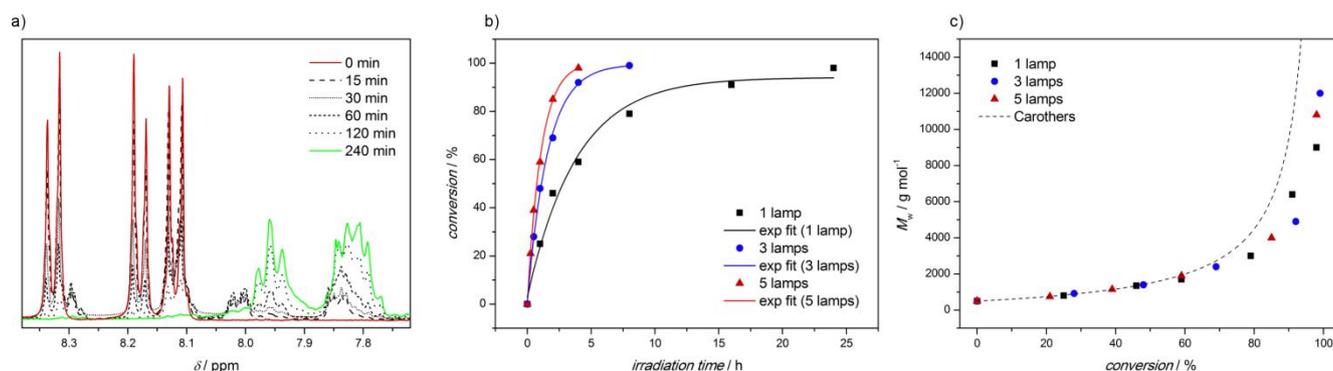
common analytical techniques (NMR, SEC, MS) was employed for each polymerization sample. Fortunately, the NITEC system is invariant to oxygen or water, as evidenced in earlier studies,[25] facilitating the sample preparation to only dissolving the crystalline monomer and sealing the crimp-top vials to avoid evaporation of the solvent. Subsequent to the irradiation of **M₂,** the solvent (THF) was removed under reduced pressure and the resulting crude polymer mixture was analyzed by size-exclusion chromatography (SEC), nuclear magnetic resonance (NMR) spectroscopy, electrospray-ionization mass spectrometry (ESI-MS), UV-Visible spectroscopy (UV-Vis), and fluorescence spectroscopy. NMR analysis demonstrated that only the desired cycloaddition occurs, excluding the existence of any side reactions (Fig. 1a). Comparing the spectra of monomer and polymer, a shift of the aromatic protons (a, b, c, d) to higher fields (a', b', c', d') can be observed. Furthermore, the resonances assigned to the fumerate double bond disappear (e), along with the appearance of the pyrazoline signals (e', e'').

SEC analysis of the crude polymer produced within 8 h using 3 UV lamps reveals a mixture of low molar mass termination product (34 – 36 min retention time) and a polymer distribution (34 – 26 min retention time) with overall molecular weight values of $M_n = 2,500$ g mol⁻¹ and $M_w = 12,000$ g mol⁻¹ (green line Fig. 1b). In order to carefully examine both types of product, the polymer was separated from the lower molar mass moieties by precipitation in cold methanol yielding 60 wt.% polymer and 40 wt.% cyclic oligomers. SEC analysis of both fractions (Fig. 1b) displays the success of this facile separation method, as the precipitating product (red line) is almost quantitatively free of oligomeric species present in the non-precipitating fraction (dashed gray line). Thus, a polypyrazoline of $M_n = 7,400$ g mol⁻¹ and $M_w = 12,900$ g mol⁻¹ could be isolated. The low molar mass material was additionally analyzed by ESI-MS in order to verify its cyclic structure (Fig. S3). The mass spectrum confirms the NMR results which suggest that open-chain oligomers containing residual tetrazole units have been converted into pyrazoline species. The m/z values perfectly match the predicted masses for cycles composed of 2, 3, and 4 monomer units.

The most appealing property of the polypyrazolines along with the novel polymerization technique presented here lies in the fluorescence of the pyrazoline moieties (Fig. 2a). The monomer

Fig. 3   a) ^{1}H NMR spectra of the raw product obtained by polymerization of **M$_2$** produced with 5 UV-lamps after different irradiation times. The displayed region contains the signals a, b, c, a' and b' (refer to Fig. 1a). b) Kinetic plot of conversion vs. irradiation time calculated by integrating the respective NMR spectra. The kinetic plot parameters (Table S2) and additional kinetic data are available in the Supporting Information (Fig. S9). c) Corresponding M_w vs. conversion plot. M_w values were obtain via SEC analysis of the crude polymerization mixtures. The Carothers equation is provided in the Supporting Information (Eq. S2).

themselves do not show any fluorescence, which is demonstrated for **M$_2$** in Fig. 2b. When the fluorescence spectrum is recorded after irradiation with UV-light, the product exhibits strong fluorescence in a broad emission spectrum ranging from 450 nm to 750 nm (Fig. 2a). A further appealing feature of the pyrazoline-based material is that, besides its biocompatibility as demonstrated in previous studies,[26] it absorbs light at longer wavelengths than 400 nm (dashed red line in Fig. 2a) and thus fluorescence can be induced at wavelengths suitable for imaging applications in living organisms.

In order to investigate the polymerization process in detail, its progress was monitored by NMR and SEC analysis, determining the conversion and the molar mass of the produced material (Fig. 3) simultaneously. In the following, the results of the photo-polymerization of **M$_2$** are being discussed exemplarily. Proton NMR spectroscopy is the ideal tool to monitor the reaction process of the current system as the signals of monomer and product can be integrated without significant overlap. The overview spectrum (Fig. 1a) which compares the signals of **M$_2$** and the polymer reveals that the pyrazoline protons (e', e'' in Fig. 1a, top) and the spacer resonances (k, k') can both serve as indicators for the conversion, as well as the aromatic protons (a, b, a', b') which are depicted in Fig. 3a (Fig. S7 and S8). Either integration approach results in conversion values within a NMR typical 5 % error. The conversion was calculated according to equation S1 (Supporting Information). Consequently, the resulting conversions were plotted against the reaction time under variation of the irradiation intensity (1, 3, or 5 lamps, each 36 W). As expected, the reaction time necessary to achieve full conversion is reduced from 24 h to 4 h when the irradiation power is increased from 36 W to 180 W. Noteworthy is the exceptional agreement of the experimental data points and an exponential fit attests a reaction of pseudo first-order kinetics for all employed irradiation intensities (Eq. S2 Fig. S9, and Table S2).

In order to obtain a molar mass vs. conversion plot (Fig. 3c) the SEC data of the crude polypyrazoline samples were evaluated (Fig. S6-S8). Again, all three different irradiation intensities were followed and the weight-average molar masses (M_w) were compared to the respective theoretical molar mass evolution given by Carothers equation (Eq. S3).[27] The values of all three

data sets correlate well with Carothers theory except for very high conversions where the molar masses of the experimental data still fit the shape of the curve, yet the experimental values are lower. This observation can be explained by the effect of the termination reaction producing low molar mass material, which lowers the overall molar mass of the polymer mixture. A termination reaction – here a ring closure – was not taken into account in the original theoretical work of Carothers.

Conclusions

In summary, we introduce an efficient light-induced polycondensation as a facile avenue to fluorescent polymers. The step-growth polymerization of newly designed photoreactive monomers – each containing a photosensitive tetrazole and a dipolarophile unit joint by a short linker – is demonstrated. In a facile-to-conduct polymerization process polypyrazolines were successfully generated at ambient temperature and atmosphere, without any catalyst. The polymerization kinetics were followed by NMR and SEC and satisfy classical Carothers conditions, despite the generation of cyclic low molar mass termination product accumulating during the polymerization process. The intense fluorescence of the final product provides a technology platform for future in-vivo imaging applications of the generated polymers.

Notes and references

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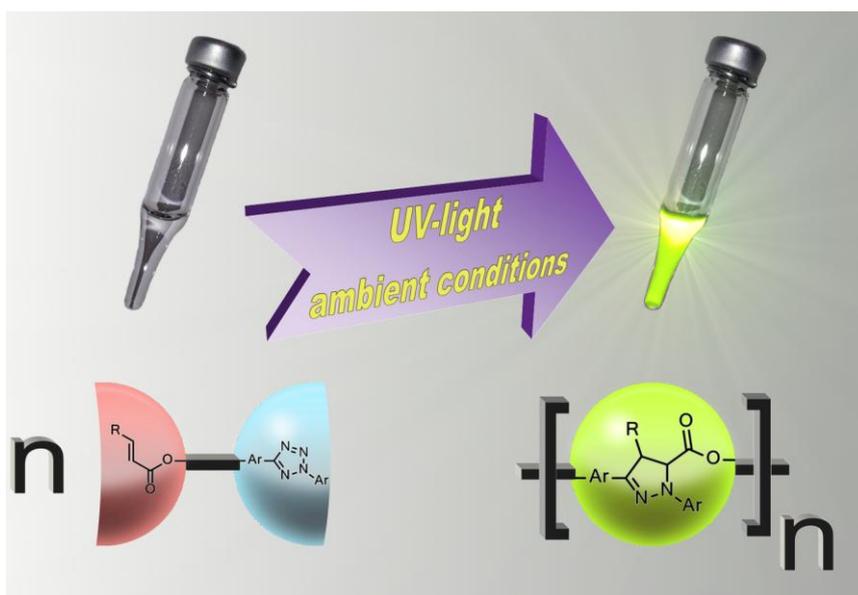10 *Germany*
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