Protection Group Free Synthesis of Sequence-Defined Macromolecules via Precision λ-Orthogonal Photochemistry

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Abstract: We report an advanced light-induced avenue to monodisperse sequence-defined linear macromolecules via a unique photochemical protocol, which does not require any protection group chemistry. Starting from a symmetrical core unit, precision macromolecules with molecular weights up to 6257.10 g mol⁻¹ are obtained via a two-monomer system: (i) a monomer unit carrying a pyrene functionalized visible light responsive tetrazole and a photo-caged UV responsive diene, enabling an iterative approach for chain growth; (ii) a monomer unit equipped with a carboxylic acid and a fumarate. Both light-induced chain growth reactions are carried out in a λ-orthogonal fashion—exciting the respective photosensitive group selectively and thus avoiding protecting chemistry. In-depth characterization of each sequence-defined chain, carried out via size-exclusion chromatography (SEC), high-resolution electrospray ionization mass spectrometry (ESI-MS) and NMR spectroscopy, confirms the precision nature of the macromolecules.

The unique class of perfect monodisperse sequence-defined oligomers emerged inspired by nature’s precision and gains ever since growing scientific interest. Such macromolecules consist of monomers or building units placed at exact positions along the polymer chain establishing monodispersity. Indeed, monomer sequence regulation plays a key role in biology and is essential for critical features of life, such as self-replication and complex self-assembly. Guided by nature, the synthesis of sequence-defined macromolecules requires non-statistical and highly efficient synthetic processes and is envisioned as the key towards highly functional materials, for instance, in molecular bar coding, biological applications in synthetic enzyme design or precision network synthesis.[1] Indeed, interesting concepts providing simple, scalable and orthogonal strategies have been introduced forming the foundation for the class of macromolecules that feature sequence-definition including peptides, peptoids and conjugated oligomers or dendrimers.[1, 2] Single unit monomer insertions (SUMIs),[2a, 3] liquid phase polymerizations[4] under bulk or flow conditions as well as solid supports[5] exploiting the iterative addition of single units or modular building blocks and exponential growth strategies,[2a, 4, 6] for instance, have been investigated. Lately, sequence-defined macromolecules draw increased attention as potential data storage medium where precise placement of functional groups is of crucial importance,[6, 7] summarized in a recent review.[8] Herein, we are introducing a versatile completely protection group free strategy for the synthesis of monodisperse sequence-defined macromolecules by combining two photosensitive groups activated in a λ-orthogonal fashion (Scheme 1). A two monomer approach is employed exploiting benzaldehydes and tetrzaoles as light responsive moieties resulting in monodisperse macromolecules as potential materials for applications in data storage or as photoresists.[10, 2a]

Our previous approaches reported a photochemical protocol relying on benzaldehydes and furan caged maleimides to afford sequence-defined macromolecules.[2c, 10] Consequently, after every chain growth step, the obtained oligomers needed to be thermally treated upon further chain extension. To reduce the amount of reaction steps that are performed with the macromolecules itself and to increase the overall yield, it is critical to avoid protection group chemistry. One option to prevent protecting groups is to introduce photolabile moieties which can be excited independently from each other by irradiation with different colors of light. A pyrene functionalized tetrazole is visible light responsive and forms a nitrile imine upon irradiation at 410 nm, which can be exploited to perform a nitrile imine-carboxylic acid ligation (NICAL).[21] α-Methyl benzaldehydes entail photo-caged UV responsive dienes which undergo [4+2] Diels–Alder (DA) cycloadditions[2c, 10] at 365 nm in presence of dienophiles such as fumarates. Combining both photoactive groups in one molecule enables light-induced chain growth reactions in a λ-orthogonal fashion by the selective excitation of the respective photosensitive group. Our herein reported synthesis of sequence-defined macromolecules is based on the convergent light-induced chain extension of a symmetrical core unit 1 employing two different monomer units in a flow system using a custom made flow reactor. First, a monomer 2 equipped with a pyrene functionalized visible light responsive tetrazole for

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chain extension via NICAL and a photo-caged UV responsive diene for DA cycloaddition and second, a monomer unit 4 carrying a carboxylic acid and a fumarate were designed to enable iterative chain growth. The synthetic strategy using two monomer building blocks enables the synthesis of perfect macromolecules avoiding protecting chemistry in-between the addition of the single monomers. Using the symmetric bifunctional carboxylic acid 1 as a core unit allows to grow sequence-defined macromolecules in a bidirectional manner, thus, enabling a significant increase of molecular weight per chain extension step. Therefore, high molecular weights can be accessed in as few reaction steps as possible by switching between the two monomer types.

Flow reactors present two significant advantages compared to conventional reaction conduction under batch conditions. First, according to the Beer-Lambert’s law, reactor tubes with a low diameter can be employed maximizing light penetration and therefore reaction efficiency. Second, reaction scaling can be performed by increasing reaction times while retaining the very same conditions in terms of volume, concentration and exposure time. Thus, we designed a flow reactor (refer to the SI) using LEDs ($\lambda_{\text{max}} = 410 – 420$ nm) as light source. As depicted in Scheme 2, the symmetrical dimer 3a (1964.28 g mol$^{-1}$, 89 %) was obtained by NICAL of the core 1 and a slight excess of the monomer 2 (2.3 eq.) in anhydrous DCM. While the tetrazole is transformed into the reactive nitrile imine at 410 nm irreversibly, the benzaldehyde moiety remains in its ground state and is unreactive. The bidirectional growth leads to extension on both chain ends resulting in sequence-defined macromolecules end functionalized with $\alpha$-methyl benzaldehyde moieties allowing further growth by DA cycloaddition. In a subsequent oligomerization step, an excess of monomer 4 (4.0 eq.) was added to the dimer 3a employing a PL-L lamp ($\lambda_{\text{max}} = 365$ nm) as light source yielding the symmetrical carboxylic acid functionalized tetramer 3b (2540.79 g mol$^{-1}$). Excess of monomer 4 and the target compound 3b (85 %) can be selectively obtained by extraction of the crude mixture. The irradiation was performed under batch conditions employing photovials as reaction vessel because the DA cycloaddition between $\alpha$-methyl benzaldehyde and fumarate is not feasible under our flow conditions, possibly because an anhydrous inert atmosphere free of oxygen cannot be established. Nevertheless, we wish to highlight that photoreactions with $\alpha$-methyl benzaldehyde and fumarate can be performed successfully in a commercially available Vapourtec flow reactor. Applying similar reaction conditions according to the dimer formation afforded the symmetric sequence-defined

![Scheme 2](image-url)
hexamer 3c (4110.69 g mol\(^{-1}\), 79 %) by chain extension of the tetramer 3b employing the UV-responsive monomer 2 in our flow setup introducing benzaldehyde moieties at the chain ends for further extension. The oligomer was further extended under batch conditions using the hexamer 3c and monomer 4, for the introduction of carboxylic acids as end groups, giving octamer 3d (4687.20 g mol\(^{-1}\), 65 %) after UV-induced cycloaddition. In the final chain growth step, the targeted symmetrical sequence-defined macromolecule 3e (6257.10 g mol\(^{-1}\), 75 %) was obtained by UV irradiation of octamer 3d with monomer 2 employing a LED light source in a flow reactor. 3e also features benzaldehydes at its chain ends which potentially enables further polymer growth towards higher molecular weight. The low overall yield can be explained by the necessary purification of the intermediates since the photoreaction demands equimolar stoichiometry and a slight excess of monomers was used. Due to their bidirectional growth, the sequence-defined oligomers require quantitative conversion of the chain ends, leading to a certain loss in overall efficiency in addition. Compared to our previous report, purification is simplified in-between the single chain extension steps because the polarity of the macromolecule is drastically changed.\(^{(2)}\) Every second extension reaction introduces carboxylic acids at the macromolecules chain ends and enables the facile separation of the single generations.

Successful formation of the targeted decamer 3e and respective intermediates was verified by SEC, high resolution Orbitrap ESI-MS and NMR. All SEC traces of the core unit 1, the monomer units 2 and 4 and the oligomers 3a-d up to the final monodisperse sequence-defined decamer 3e are depicted in Figure 1a. The molecular weights were obtained relative to a polystyrene calibration. Inspecting the SEC traces at each synthetic step, a shift towards increased molecular weight can be observed from the bifunctional core unit 1 as well as the monomers 2 and 4 to the iteratively generated sequence-defined macromolecules 3a-e. The traces resulting from the sequential approach confirm the purity of the oligomers and the increase in molecular weight verifies the successfully conducted synthetic strategy using different wavelengths of light to initiate chain growth. The chromatographically determined dispersities of \(D = 1.01\) for all in Figure 1a depicted SEC traces representing the building units and oligomers are evidencing the monodisperse nature of the synthesized sequence-defined macromolecules 3a-e. In addition, SEC analysis verifies the iterative chain extension by switching between the two monomer types with an increase of molecular weight after the addition of the respective monomers. In Figure 1b, the high resolution ESI-MS spectrum of the targeted symmetrical sequence-defined macromolecule 3e is depicted in the m/z range from 1500 to 5000. The recorded spectrum only reveals mass signals of respective charged sodium adducts at \(m/z = 3151.3119\) \([\text{3e}\text{-2Na}]^{+}\), \(m/z_{\text{calc}} = 3151.2924\), \(\Delta m/z = 0.0195\)), \(m/z = 2108.5312\) \([\text{3e}\text{-3Na}]^{+}\), \(m/z_{\text{calc}} = 2108.5247\), \(\Delta m/z = 0.0065\)) and \(m/z = 1587.1443\) \([\text{3e}\text{-4Na}]^{+}\), \(m/z_{\text{calc}} = 1587.1408\), \(\Delta m/z = 0.0035\)) all assigned to the oligomer 3e. Recorded mass signals are in excellent agreement with calculated molecular mass. In addition, Figure 1c shows the experimental and simulated isotopic pattern of the double charged species 3e containing two sodium counter ions in excellent agreement. The characterization via SEC, the full mass spectrum and the conformity of recorded and calculated isotopic pattern clearly evidences monodispersity and the successful synthesis of the sequence-defined decamer 3e (full MS spectra and isotopic patterns of 3a-d can be found in the SI).

Further characterization was carried out via NMR spectroscopy. The proton spectrum and structure of the decamer 3e are depicted with signal highlighting in Figure 2. Intramolecular rearrangement after NICAL formation from a hydrazonic anhydride to a hydrazide (see Supporting Information) results in a complex proton spectrum. Typical signal broadening, generally observed for macromolecules, and the formation of different isomers after DA cycloaddition further complicates the analysis via NMR. Nevertheless, characteristic resonances can be assigned to the targeted oligomer 3e. Most importantly, a proton resonance detected at 10.73 ppm (a) resulting from the benzaldehyde moiety implies the successful chain extension employing monomer 2 and the option for further chain growth. Broad singlets at 11.06 and 10.84 ppm (a) additionally confirm the
formation of hydrazonic anhydride and hydrazide after conducting the NICAL reaction. The introduction of pyrenes into the backbone of the sequence-defined oligomers can be verified by proton resonances ranging from 8.80 to 7.50 ppm. Resonances detected between 5.60-4.90 (d) and 3.50-2.50 ppm (e) indicate successful formation of the tetrahydroanthalene structure formed by DA cycloaddition (detailed resonance assignments can be found in the SI, refer to Figures S52 and S55).

**Figure 2.** $^1$H-NMR spectrum of the obtained decamer 3e recorded in CDCl$_3$ at 400 MHz. Characteristic signals are highlighted and refer to the depicted structure.

In summary, we pioneer a photochemical protocol carried out in a $\lambda$-orthogonal fashion and thus avoiding protection chemistry for the synthesis of monodisperse precision sequence-defined macromolecules with a molecular weight up to 6257.10 g mol$^{-1}$. A symmetrical bifunctional carboxylic acid was chosen as a core unit enabling bidirectional chain growth and thus providing a significant increase of molecular weight per chain extension step. Thus, symmetrical sequence-defined molecules were obtained using a monomer unit carrying two photosensitive groups as key molecule, which can be selectively activated for chain extension by exposure to UV light at disparate wavelengths without protecting groups. Consecutive chain growth employing a complementary monomer led to the desired symmetric sequence-defined macromolecules in an iterative approach. Successful formation of the macromolecules was evidenced by in depth characterization employing SEC, ESI-MS and NMR. The evolution of molar mass obtained via SEC, proton NMR, recorded mass spectra and the conformity of recorded and calculated isotopic pattern underpin the precision nature of the macromolecule, with monodisperse character and sequence definition. Thus, the efficiency of our $\lambda$-orthogonal photochemical protection group free avenue has been established.

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**Conflict of Interest:** The authors declare no conflict of interest.

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No protection needed: A strategy employing a highly efficient \( \lambda \) - orthogonal photochemical protocol by selective excitation of photosensitive groups paves the way to precision monodisperse sequence-defined linear macromolecules without any need for protection group chemistry. The chain growth is based on a two-monomer system enabling protection group free convergent chain extension on-demand.