Photo-induced sequence defined macromolecules *via* hetero bifunctional synthons†

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We report the first photochemical protocol for the generation of sequence defined macromolecules employing two hetero bifunctional photoreactive synthons, exploiting the orthogonal nature of photochemical – *via* the use of caged dienes – and thermally driven ligation protocols. We demonstrate that the iterative alternating synthon addition to an initial bifunctional core under irradiation at ambient temperature enables the generation of a macromolecule with up to 10 units (M = 3231.58 g mol^{-1}, $Đ$ = 1.00). The resulting macromolecules are monodisperse and feature absolute chain end fidelity. The unit-by-unit construction of the macromolecule is evidenced by Nuclear Magnetic Resonance Spectroscopy, Electrospray Ionization Mass Spectrometry and Size Exclusion Chromatography. The fundamental principle demonstrated herein paves the way for employing photochemical strategies for the design of sequence defined polymers.

Achieving sequence control in synthetic polymers is considered as one of the most challenging tasks in contemporary macromolecular chemistry.[1,2] In the past years, several synthetic avenues have been followed, some relying on effectively generating multi-block copolymers with short individual block lengths by carefully optimizing reversible deactivation polymerization protocols, *e.g. via* Reversible Addition Fragmentation chain Transfer (RAFT) polymerization[3–5] or Cu(0) mediated polymerization,[6–9] as well as time-controlled sequential polymerization.[10–12] Despite the elegance of these approaches, the obtained macromolecules suffer from either an inherent dispersity associated with the individual polymer blocks, a very short sequence length or both.[13] The provision of truly sequence defined polymers has been recently advanced by promising innovations – although based on highly demanding synthetic strategies with elaborative purification protocols – some exploiting templates,[14] DNA templates,[15–17] solid supports[18] or even complex biomimetic systems such as molecular machines.[19] Only few examples describes sequence defined macromolecules synthesized in a simple and direct manner, *e.g. via* copper assisted alkyne-azide cycloaddition[20] or small molecule multi-component reactions.[21]

A facile method to generate high molecular weight sequence defined macromolecules, inspired by efficient modular ligation chemistry concepts,[22,23] would advance the field of macromolecular chemistry to elaborate fully spatially controlled synthetic proteins, enzymes or DNA mimics. Herein, we present a novel concept for the generation of sequence defined synthetic macromolecules based on the use of hetero bifunctional photoreactive molecular synthons as tools for defined macromolecular design. Indeed, photochemically induced reactions have thus far not been exploited for the provision of sequence defined macromolecules, despite the inherent advantages these reactions may provide, including operation at ambient temperature and quantitative yields under equimolar reaction conditions as well as spatial and temporal control.[23,24] Our team has recently exploited the photochemical provision of caged dienes and dienophiles from photoenol and phenacylsulfide precursors in modular Diels–Alder reactions[23] for the design of complex macromolecular architectures[25–28] as well as spatially resolved surface modification.[29] Scheme 1 illustrates the synthetic pathway taken in the current approach for the generation of a sequence defined macromolecule based on the aforementioned photoreactive species.

Both hetero bifunctional monomers, synthons **1** and **2**, carry a photoreactive unit, *i.e.* a photoenol or a phenacylsulfide respectively, and a diene or dienophilic unit. It should be emphasized that the judicious combination of the reactive groups is of key importance: the open chain diene is combined with the photoenol species **1**, whereas the dienophile releasing phenacylsulfide is combined with a dienophile (furan protected

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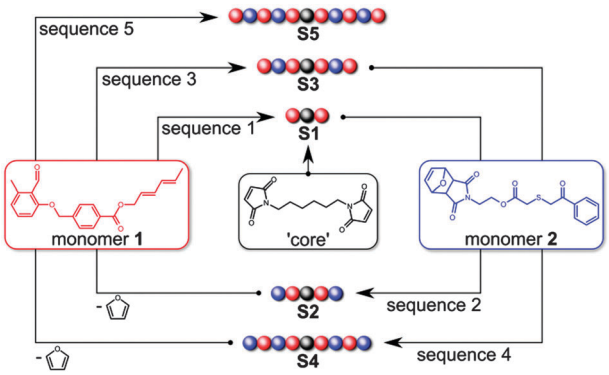

Scheme 1 Overview of the iterative synthesis of the sequence defined macromolecule **S5** based on orthogonal molecular synthons: **1** reacts with the core in the first sequence to provide **S1**, then reacting with synthon **2** in the second sequence to provide **S2**. Subsequent to the deprotection steps, the cycle is repeated up to the fifth sequence.

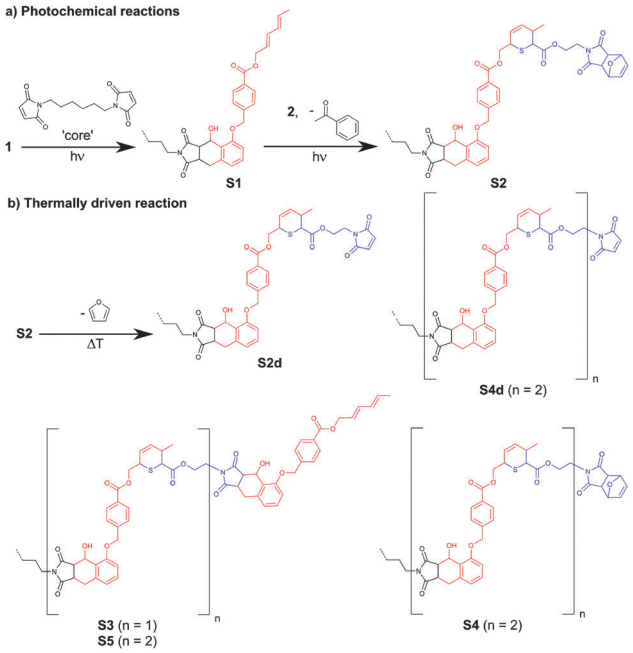

Fig. 1 Synthetic pathway for the photochemical construction (a) of the sequence defined polymer and intermediate retro-Diels–Alder (rDA) deprotection (b). For clarity, only one part of the synthesized symmetrical molecules is represented.

maleimide function) **2**. The synthetic key that makes the current concept viable is the inherent orthogonality of photo-triggered and thermally induced Diels–Alder reactions, *i.e.* the non-reactive presence of an open chain diene and an activated dienophile that do not undergo a ligation process, and are separately addressable *via* photochemical approaches. The attractiveness of the employed route to both synthons (esterification) with the dienophilic/diene unit is its simplicity and versatility. Both criteria suggest the potential diversity of chemical structures in monomer design. For instance, selected side functions can be introduced into the design on demand or molecules can be employed – *via* the inherently reactive chain end functionality of the resulting macromolecules – for grafting reactions. By employing trilinkers, the linear sequence defined macromolecule may be transformed into 3D networks with a completely controlled topology.

The sequence commences by reacting synthon **1** with the bifunctional maleimide core *via* irradiation with a lamp featuring a wavelength maximum of λ_{max} = 350 nm. The photoligation[25] leads to the first product **S1**. Importantly, this first photoreaction is orthogonal with regard to a potential thermally driven Diels–Alder ligation between the maleimide functions with the open chain diene groups of **1** (refer to the ESI† section). The dimer **S1**, featuring two open chain diene functionalities as chain termini, reacts in a subsequent photoreaction (employing the same lamp) with synthon **2**, involving an *in situ* highly reactive dienophile (thioaldehyde)[28] to afford the tetramer **S2**, and providing a molecule of acetophenone (Fig. 1a). The thermal deprotection of **S2** *via* a retro-Diels–Alder (rDA) reaction leads to the product **S2d**, bearing two maleimide chain termini (Fig. 1b), which are able to react under the same conditions as in the first sequence with synthon **1**. The described cycle is repeated in order to obtain the third sequence product **S3** (hexamer), which is reacted with synthon **2** in the fourth sequence to generate **S4**. **S4** undergoes a rDA reaction as described for **S2** in order to afford the deprotected maleimide chain ended **S4d**. Again, the photoreaction with **1** is repeated to generate the fifth sequential product **S5**, featuring a total of 10 subsequently incorporated units.

The ^{1}H NMR spectra of the novel bifunctional photoreactive species **1** and **2** are depicted in Fig. 2, respectively. Synthon **1** features – on the one hand – a photoreactive moiety, *i.e.* photoenol, evidenced *via* the resonances associated with protons m and n (2.51 and 10.67 ppm, respectively) and – on the other hand – an open chain diene, *i.e.* sorbyl group, associated with the resonances of protons c, d and f (6.01, 6.27 and 4.75 ppm, respectively). Similarly for **2**, the resonances of protons related to the photoreactive moiety, *i.e.* the phenacyl-sulfide group, are recognizable (g to j protons), as well as those connected with the protected maleimide (protons a and b located at 6.42 and 5.18 ppm respectively). In the following, the cited protons c and d from synthon **1** are labelled 1c and 1d. The same convention is employed for the protons m and n (1m and 1n), as well as for the protons a, b and c from synthon **2** (2a, 2b and 2c).

Following the synthetic pathway, the first sequence to afford **S1** (Fig. 1a) after the reaction of the bifunctional maleimide core with monomer **1** was successful. The ^{1}H NMR spectrum of **S1** depicts the success of the photoreaction, since the resonances of the photoreactive moiety (protons 1m and 1n) disappear and are shifted according to the newly formed covalent bonds upon reaction with the core (refer to Fig. S13 to S16, ESI†). Importantly, the unreacted sorbyl groups of **1** are still present, and the resonances assigned to the protons 1c, 1d and 1f remain unchanged (Fig. 3a, top), implying that the photoreaction between the maleimide and photoenol groups is orthogonal without interfering with the sorbyl groups.

A detailed analysis *via* ^{1}H NMR demonstrating the orthogonal nature of the sorbyl end groups in the reaction mixture under

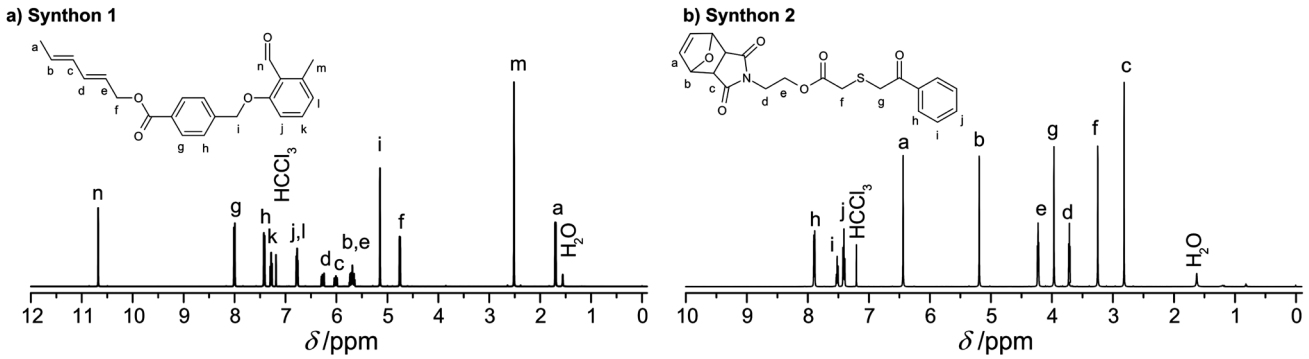

Fig. 2 ^{1}H Nuclear Magnetic Resonance (NMR) spectra of the two hetero bifunctional photoreactive monomers **1** (a) and **2** (b) (500 MHz, $CDCl_3$).

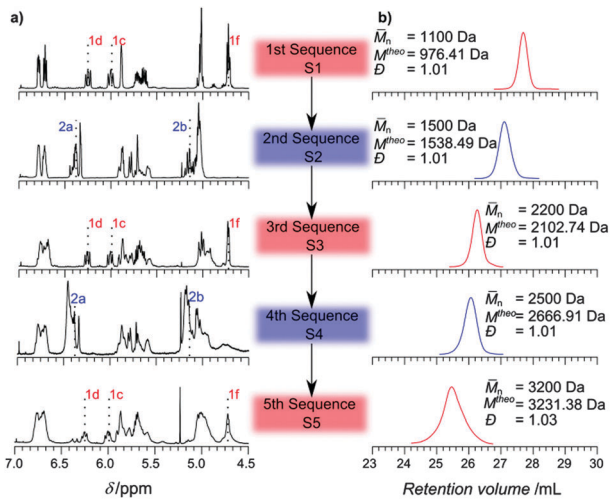

Fig. 3 Evolution of the ^{1}H Nuclear Magnetic Resonance (NMR) spectra (a) and size Exclusion Chromatography (SEC) traces (b) of the sequence defined products **S1** to **S5**. (a) The NMR resonance of the key protons of synthon **1** (1c, 1d and 1f) and **2** (2a and 2b) are shown (500 MHz, $CDCl_3$). (b) SEC performed in THF (PS calibration).

different thermal conditions is included in Fig. S12 (ESI†). For the second sequence (product **S2**), the sorbyl termini of dimer **S1** react with the *in situ* formed thioaldehyde, leading to the disappearance of the resonances assigned to the protons 1c, 1d and 1f, shifted according to the newly created covalent bonds (refer to Fig. S19 to S21, ESI†), and the presence of the unreacted protected maleimide group of **2** with the protons 2a and 2b (Fig. 3a, second spectrum from the top). Subsequent to the successful furan cleavage (refer to Fig. S23 and S24, ESI†), an iteration of both photoreactions is carried out, leading to **S3** (photoreaction between **S2d** and **1**) and **S4** (photoreaction between **S3** and **1**). Once more, the iteration of the furan cleavage (leading to **S4d**, refer to Fig. S27, ESI†) and a subsequent photoreaction with synthon **1** lead to **S5**. After the odd ordered sequences (third and fifth), the proton resonances of the sorbyl termini (1c, 1d and 1f) are observed as is the case for the first sequence resulting in **S1** (Fig. 3, further details available in Fig. S25 and S28, ESI†). Similarly, after the even ordered sequence (*i.e.* fourth), the proton resonances of the furan protected maleimide termini from **S4** (2a and 2b) are observed analogous to **S2** generated after the second sequence (Fig. 3, further details available in Fig. S26, ESI†). The presence of the alternating resonances of the chain termini of 1c, 1d and 1f, on the one hand, and 2a and 2b on the other hand, confirm the success of the sequence defined synthesis, reflecting the alternating addition of monomer **1** and **2** to the starting core. A more detailed analysis of the signal assignment of the end groups and the constituting backbone is included in the ESI† section *via* two-dimensional NMR spectroscopy.

A further analysis of the sequence synthesized molecules *via* SEC additionally confirms the success of the proposed procedure to generate highly defined macromolecules. Along the ^{1}H NMR spectra depicted in Fig. 3, the corresponding SEC traces of **S1** to **S5** are shown (Fig. 3b). The average molecular weight (M_n) of each sequence product is increasing (retention volume decreasing) from 1100 g mol^{-1} for **S1** to 3200 g mol^{-1} for **S5**. Despite the non-universal character of SEC for determining the molecular weight (necessity of calibration), the reported M_n are surprisingly accurate values (reported in Fig. 3), closely corresponding to the theoretical molecular weight (M^{theo}) of each product. The increase of the molecular weight is in complete agreement with the increasing size of the sequence defined macromolecule. Importantly, the dispersity ($Đ$) which is close to 1 (reported 1.01 until **S4d**) underpins the monodispersity of each sequential product, which is below unambiguously demonstrated by mass spectrometry. This observation highlights the unique character of the designed macromolecule **S5**, as well as of lower order products, and thus the precision of the applied strategy.

The monodisperse nature of the sequence defined products is evident with the final characterization performed *via* ESI-MS. Fig. 4 depicts an overlay of ESI-MS spectra from **S1** to **S3**, each featuring a single molecular ion peak.

In the presence of a counter ion, the evolution of the molecular weight after the consecutive reactions enables to accurately observe the addition of the expected synthon fragments, showing that the monodisperse character of the sequence defined products remains unchanged. The first reported mass of 1260.34 m/z for **S1** is in complete agreement with the theoretical value of the single charged molecule with the counter ion (1260.75 m/z^{theo}, further details in the Table S5, ESI†) obtained from the bifunctional maleimide core ligated to two molecules of synthon **1**. Detailed ESI-MS spectra with the simulated isotopic pattern of the expected molecules, as well as

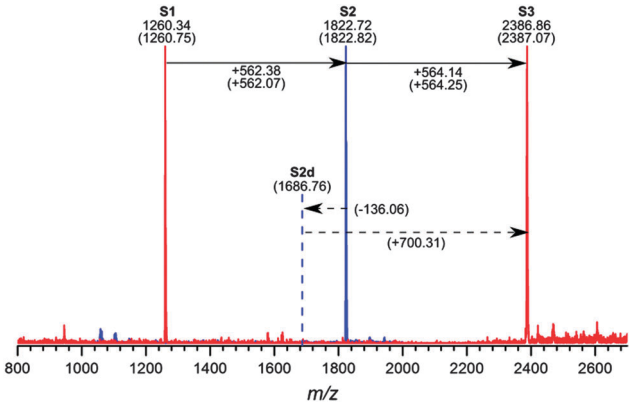

Fig. 4 Electrospray ionization mass spectra (ESI-MS) of the sequence products **S1** to **S3** (theoretical values in brackets). For clarity, the theoretical peak for **S2d** is provided (see text).

Collision Induced Decay (CID) experiments are described in the ESI† section. Subsequent to the second sequence, the mass increases by 562.38 m/z, corresponding to two additional furan protected maleimide fragments from synthon **2** (562.07 m/z^{theo}). The mass shift for the third sequence from **S2** to **S3**, *via* **S2d** (shown by the dashed line in Fig. 4) corresponds to the cleavage of two furan groups (136.06 m/z^{theo}), and the gain of two molecules of synthon **1** (700.31 m/z^{theo}), leading to a total of 564.14 m/z experimentally and 564.25 m/z (= −136.06 + 700.31 m/z) theoretically. Unfortunately, the higher ordered sequences **S4** and **S5** could not be characterized *via* ESI-MS, due to their high molecular weight. However, mass-spectrometry conducted with Matrix Assisted Laser Desorption Ionization (MALDI) enabled the identification of the molecular ion peak for **S5** (refer to Table S6, ESI†). The accuracy of the presented results (mass differences from 0.10 to 0.41 m/z between the experimental and theoretical values) fully correlates the observed mass shifts with the conducted reactions, *i.e.* the first and third sequences leading to the addition of two synthons **1**, and the second sequence to two additional fragments from synthon **2**.

In summary, a conceptually new synthetic platform based on photoligation is introduced to access sequence defined macromolecules at ambient temperature. The orthogonality of the proposed photoreactive system based on complementary dienophile/diene synthons enabled to systematically introduce alternating groups at the chain ends, allowing the sequence order (the molecular weight) to gradually increase. The unit-by-unit synthesized products were isolated after each sequence (up to the fifth order) and characterized, hence evidencing the validity of the proposed strategy, and underlining the specific properties of such compounds related to their exactly defined nature. Importantly, the products feature a monodisperse character and absolute chain end fidelities. We have thus proven that photochemical ligation protocols are viable tools for generating sequence defined macromolecules, holding substantial promise for the field.

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