

λ -Orthogonal Pericyclic Macromolecular Photoligation**

Kai Hiltebrandt, Thomas Pauloeuhl, James P. Blinco, Katharina Linkert, Hans G. Börner, and Christopher Barner-Kowollik*

Abstract: A photochemical strategy enabling λ -orthogonal reactions is introduced to construct macromolecular architectures and to encode variable functional groups with site-selective precision into a single molecule by the choice of wavelength. λ -Orthogonal pericyclic reactions proceed independently of one another by the selection of functional groups that absorb light of specific wavelengths. The power of the new concept is shown by a one-pot reaction of equimolar quantities of maleimide with two polymers carrying different maleimide-reactive endgroups, that is, a photoactive diene (photoenol) and a nitrile imine (tetrazole). Under selective irradiation at $\lambda = 310\text{--}350\text{ nm}$, any maleimide (or activated ene) end-capped compound reacts exclusively with the photoenol functional polymer. After complete conversion of the photoenol, subsequent irradiation at $\lambda = 270\text{--}310\text{ nm}$ activates the reaction of the tetrazole group with functional enes. The versatility of the approach is shown by λ -orthogonal click reactions of complex maleimides, functional enes, and polymers to the central polymer scaffold.

The synthesis of polymers with tailored material properties has been revolutionized with the advent of reversible

deactivation methods.^[1] Such polymers find widespread applications in areas such as drug delivery,^[2] self-healing materials,^[3] organic solar cells (OSCs),^[4] and microelectronics.^[5] Click chemistry is also an excellent method for the synthesis of macromolecular materials.^[6] The concept of click chemistry was introduced by Sharpless in 2001.^[7] Fast reaction kinetics, quantitative yield, stereoselectivity, equimolarity, orthogonal reactivity and no purification of the obtained products are unique features of click chemistry.^[8] Nevertheless, thermally induced click reactions lack spatial and temporal control. Light-triggered click reactions offer a possibility to overcome these disadvantages.^[9] In general, photochemistry is environmentally friendly, can be orthogonal, and offers the possibility of uphill photosensitization.^[10] Herein we define λ -orthogonality of click reactions as the (kinetic) preference of one reaction channel by using a specific wavelength regime. Importantly, we wish to impart this orthogonality using cheap, simple, and readily available radiation sources.

There are examples of wavelength-dependent reactions; for example, Del Campo et al. synthesized different photoactive protecting groups consisting of aromatic chromophores. These photoactive groups were then attached to surfaces. After irradiating one of these chromophores with an appropriate wavelength, small aromatic molecules and carbon dioxide are released.^[11] Franking et al. demonstrated the wavelength-dependent (254 nm vs. 350 nm) photochemical grafting of a set of alkenes onto single-crystal TiO₂ samples.^[12] Inui et al. addressed the wavelength-dependent cleavage of azirine rings forming nitrile ylides at $\lambda > 300\text{ nm}$ and acetonitrile oxides at $\lambda = 300\text{ nm}$ in the presence of oxygen.^[13] All the above studies either successfully establish the photo-triggered attachment of molecules onto surfaces, the release of small molecules from surfaces by irradiation, or the decomposition of photoactive molecules into smaller fragments. Until now, however, there is no description of a chemical system that employs orthogonal wavelength-dependent pericyclic click reactions in a one-pot fashion. The key to achieve orthogonality in photo-induced click reactions is to choose appropriate moieties that are kinetically preferred by a specific wavelength as illustrated in Scheme 1.

Initial kinetic studies of two polymers each carrying a different photoactive endgroup were performed in the presence of maleimide in varying ratios. A poly(ethylene glycol) methyl ether (PEG) chain is capped with 4-((2-formyl-3-methylphenoxy)methyl)benzoic acid ("photoenol", a diene that can be photoactivated) to form compound **1** (Supporting Information, Figure S3) and capped with 4-(2-phenyl-2H-tetrazol-5-yl) benzoic acid ("tetrazole", a nitrilamine that can be photoactivated) to form compound **2** (Supporting Information, Figure S4). Choosing the appropriate irradiation

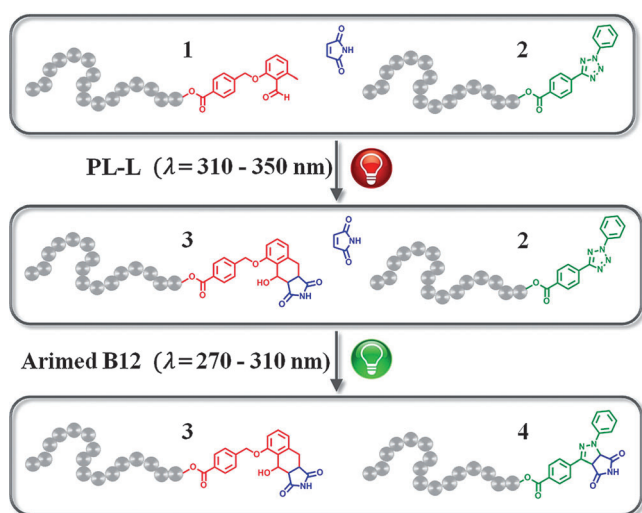
[*] K. Hiltebrandt, Dr. T. Pauloeuhl, Prof. Dr. C. Barner-Kowollik
Preparative Macromolecular Chemistry
Institut für Technische Chemie und Polymerchemie
Karlsruhe Institute of Technology (KIT)
Engesserstrasse 18, 76128 Karlsruhe (Germany)
and
Institut für Biologische Grenzflächen (IBG)
Karlsruhe Institute of Technology (KIT)
Hermann-von-Helmholtz-Platz 1
76344 Eggenstein-Leopoldshafen (Germany)
E-mail: christopher.barner-kowollik@kit.edu

Dr. T. Pauloeuhl
Current address: Institute for Complex Systems
Eindhoven University of Technology
Post Office Box 513, 5600 MD Eindhoven (The Netherlands)
Dr. J. P. Blinco, Prof. Dr. C. Barner-Kowollik
School of Chemistry, Physics and Mechanical Engineering
Queensland University of Technology (QUT)
2 George St, Brisbane, Queensland 4001 (Australia)

K. Linkert, Prof. Dr. H. G. Börner
Laboratory for Organic Synthesis of Functional Systems
Humboldt-Universität zu Berlin
Brook-Taylor-Strasse 2, 12489 Berlin (Germany)

[**] C.B.K. acknowledges continued funding from the Karlsruhe Institute of Technology (KIT) in the context of the Helmholtz STN program. K.H.'s PhD studies are funded by the Fonds der Chemischen Industrie (FCI). H.G.B. acknowledges funding from the ERC (ERC 305064).

Supporting information for this article is available on the WWW under <http://dx.doi.org/10.1002/anie.201410789>.



Scheme 1. Evidencing λ -orthogonal pericyclic reactions. The system consists of a photoenol derivative **1**, a tetrazole derivative **2**, and a dienophile such as maleimide in a true one-pot system. Irradiating the system with the PL-L lamp selectively affords the photoenol cycloadduct **3**. Subsequently, compound **2** can be transformed into compound **4** with the Arimed B12 lamp.

wavelength leads to λ -orthogonal reactivity. Both the photoenol and the tetrazole exhibit an intensive absorption in the range between 250 and 290 nm. The photoenol shows a further, less-intense absorption between 290 and 360 nm. Within this absorption range, the photoenol can be excited selectively since the tetrazole moiety only has a negligible absorption in the range between 310 and 350 nm (Figure 1). The discrete absorption enables the photochemical orthogonality between the photoenol and the tetrazole and leads to an orthogonal light-triggered reactivity with dienophiles. However, orthogonality between photoenol and tetrazole can only be achieved when the photoenol is activated prior to the tetrazole compound.

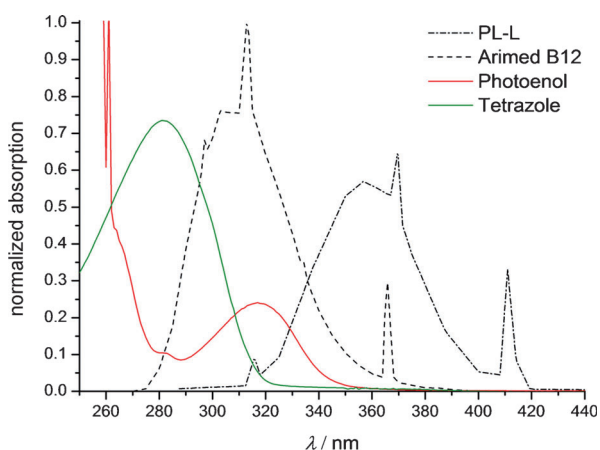


Figure 1. UV/Vis absorption spectrum of photoenol **1** and tetrazole **2** superimposed with the emission spectra of the PL-L lamp ($\lambda_{\text{max}} = 370$ nm) and the Arimed B12 lamp ($\lambda_{\text{max}} = 310$ nm). The absorption area of tetrazole has negligible overlap with the PL-L emission area, whereas the photoenol can be activated in the wavelength range of 310–350 nm by the PL-L lamp.

To achieve irradiation in these discrete regions, the compact fluorescent lamp PL-L with an absorbance maximum at 370 nm for activating the photoenol-capped polymer and the compact fluorescent lamp Arimed B12 (Supporting Information, Figure S1) with an absorbance maximum of 310 nm for activating polymers carrying the tetrazole component were employed. When the photoenol or tetrazole component are activated by the respective UV lamps, a maleimide acting as dienophile will then undergo selective reaction with only the activated species (Scheme 1).

In the first kinetic study, eight samples containing an equimolar mixture of **1** and **2** and a 10.7 equiv excess of maleimide in dichloromethane were irradiated with the PL-L lamp for various times from 0 to 15 min (see the Supporting Information). Next, six samples containing the same mixture of **1**, **2**, and maleimide were all initially irradiated for 15 min with the PL-L lamp and subsequently with the Arimed B12 lamp for 0 to 240 min (see the Supporting Information). To determine the ratios of each species, the samples were analyzed by electrospray ionization mass spectrometry. The evolution of the relative intensities of the substances **1**, **2**, **3**, and **4** in the mass spectrum were compared after the irradiation with the PL-L lamp and the Arimed B12 lamp, respectively (Figure 2; the complete procedure for the determination of the kinetic data is described in detail in the Supporting Information). The kinetic data of the samples that were irradiated only with the PL-L lamp (Figure 2a) displays an exponential decay of the photoenol capped PEG **1** in combination with the exponential increase of the corresponding cycloadduct **3**. After an irradiation time of 15 min with the PL-L lamp, the conversion is quantitative. Furthermore, the orthogonal transformation of **1** into **3** after an irradiation time of 15 min was traced by ^1H NMR spectroscopy (Supporting Information, Figure S26) in excellent agreement with the MS data. The decay of **1** follows pseudo first-order kinetics, giving a rate coefficient of $(8.88 \pm 0.37) \times 10^{-3} \text{ s}^{-1}$ under the chosen conditions (Supporting Information, Figure S28a). Furthermore, a very slow increase of the tetrazole-capped PEG **2** in combination with a slow decrease of the corresponding cycloadduct **4** is detectable. After 15 min of irradiation with the PL-L lamp, less than 3% of compound **2** were converted into compound **4**. However, if the tetrazole is irradiated under identical conditions with the Arimed B12 lamp for 15 min, a significantly higher conversion results than with the PL-L lamp (4% vs 30% determined by ^1H NMR; Supporting Information, Figure S27). Thus, the addition of the maleimide moiety to an equimolar mixture of the photoenol containing polymer **1** and the tetrazole capped polymer **2** is rapid and highly orthogonal. The mass spectra reveal that maleimide was selectively added to compound **1**, whereas the conversion of compound **2** within this one-pot reaction is negligible. The kinetic data of the remaining 6 samples that were first irradiated with the PL-L lamp for 15 min and subsequently with the Arimed B12 lamp at variable times (Figure 2b) display the exponential decay of the tetrazole capped PEG **2** in combination with the exponential increase of the corresponding cycloadduct **4**. After an irradiation time of 240 min with the Arimed B12 lamp, compound **2** is quantitatively converted into compound

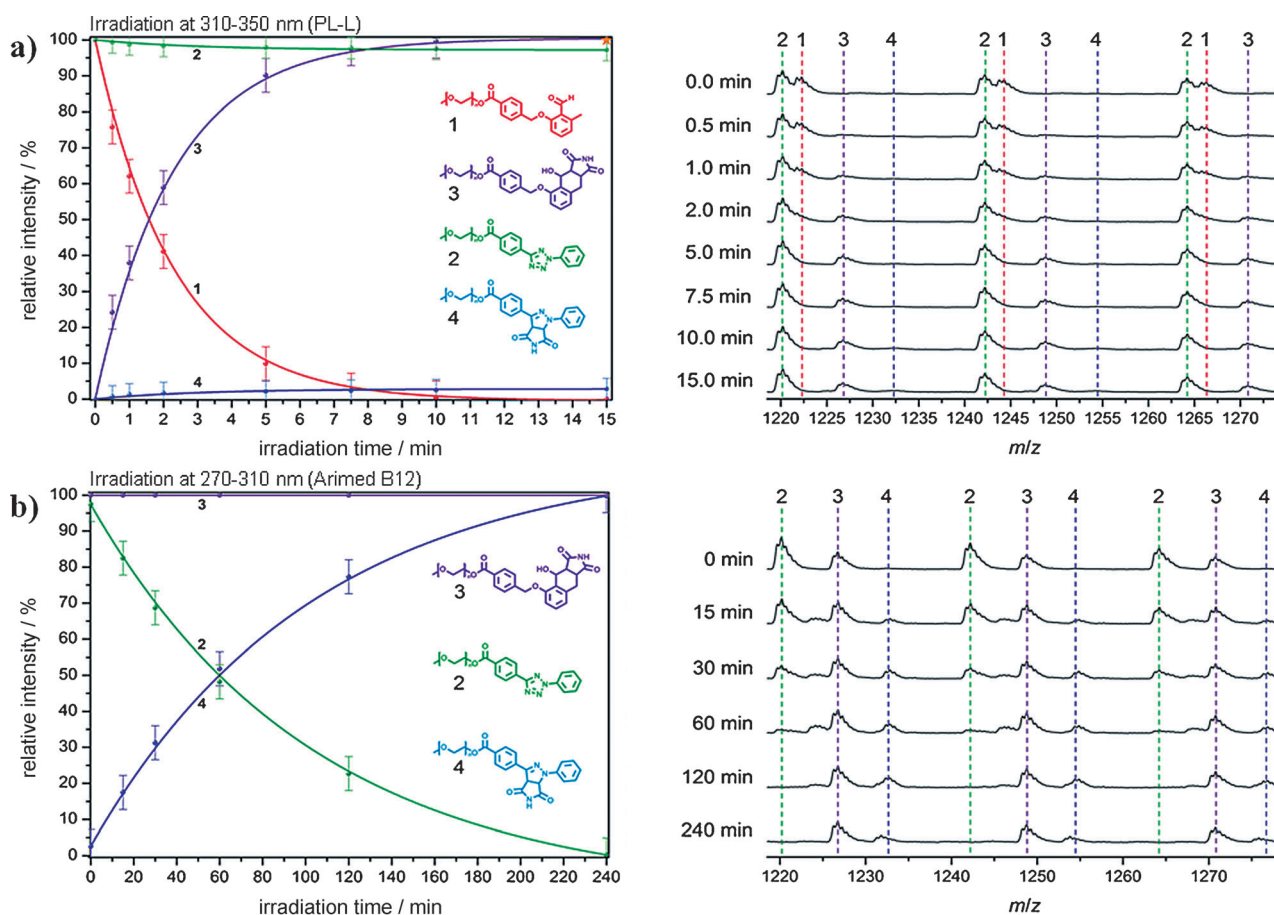


Figure 2. Kinetic data plot showing the evolution of the relative intensities of **1** (1 equiv), **2** (1 equiv), and maleimide (10.7 equiv) when irradiated following the sequence depicted in Scheme 1. A zoom ($m/z = 1218.50 - 1278.50$) of the mass spectra in the double charged region is included for each data point in the kinetic plot: a) eight samples were irradiated at $\lambda = 310-350$ nm for 15 min. The intensity of the mass spectrum signal of **1** decreases subsequently in combination with a signal intensity increase of **3**. The signal intensity of **2** decreases negligibly. The quantitative conversion of **1** into **3** was also determined by ^1H NMR spectroscopy and is marked in the kinetic plot by the orange star. b) All six of the samples were initially irradiated at $\lambda = 310-350$ nm for 15 min. The kinetic data plot was determined by irradiating the six samples at $\lambda = 270-310$ nm for 240 min. A decrease in the relative abundance of **2** and an increase of cycloadduct **4** is observed. Compound **1** was completely converted into compound **3** prior to irradiation at $\lambda = 310-350$ nm.

4. The decay of **2** follows first-order kinetics, giving a rate coefficient of $(2.03 \pm 0.02) \times 10^{-4} \text{ s}^{-1}$ under the chosen conditions (Supporting Information, Figure S28b). A key criteria of polymer click chemistry is equimolarity.^[14] Thus, a second kinetic study was performed under the same conditions (that is, sequential irradiation of a solution containing **1** and **2** in equimolar ratios with the PL-L lamp and subsequent irradiation of the solution with the Arimed B12 lamp at different irradiation times). However, the relative amount of maleimide was reduced from 10.7 equiv close to 2 equiv (Supporting Information, Figure S24), providing one equivalent of maleimide for each photoactive end group. The decay of **1** and **2**, respectively, follows pseudo first-order kinetics, affording a rate coefficient for **1** of $(7.96 \pm 0.21) \times 10^{-3} \text{ s}^{-1}$ and for **2** of $(1.92 \pm 0.14) \times 10^{-4} \text{ s}^{-1}$ under the chosen conditions (Supporting Information, Figure S25). The data clearly evidence that a system consisting of an equimolar ratio of a photoenol-capped polymer and a tetrazole-capped polymer is able to react selectively with a maleimide when the system is initially activated in the range of 310–350 nm.

The second fundamental aspect of λ -orthogonal click chemistry is demonstrating the λ -orthogonal principle on a macromolecular dilinker carrying λ -orthogonal functions at each chain end. Initially, λ -orthogonality was demonstrated by the selective addition of maleimide towards the α,ω -functional dilinker **9**. A mixture of **9** with a 2.5 equiv excess of maleimide in dichloromethane was irradiated for 15 min with the PL-L lamp. One sample was subsequently irradiated for 4 h with the Arimed B6 lamp. The molecules **9**, **12**, and **13** were analyzed by ESI-MS as illustrated in Figure 3. The ESI-MS data show that **9** was completely converted into **12** after the first irradiation and only a small amount of **13** was formed (3% conversion). The second irradiation step leads to a complete conversion into **13**.

The λ -orthogonality principle was above demonstrated for the site-specific attachment of maleimide in a system of two photoactive polymers as well as a dilinker system. Highly important, however, is the selective λ -orthogonal attachment of enes with variable and complex chemical functionality to the α,ω -functional dilinker, demonstrating the wide applic-

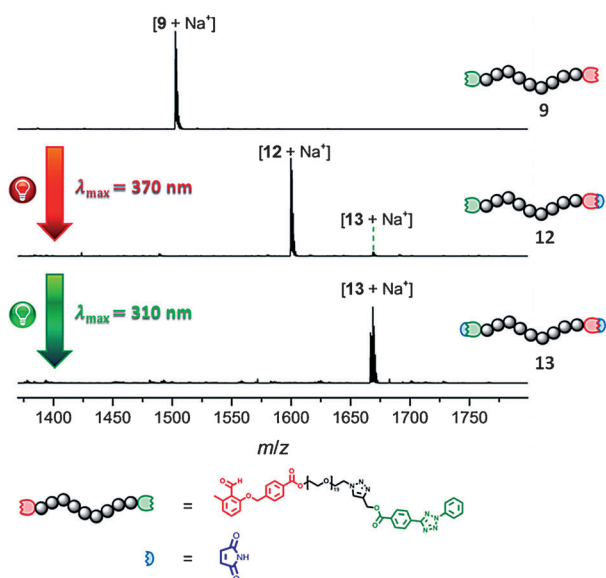


Figure 3. A system consisting of the dilinker **9** and maleimide was irradiated for 15 min at $\lambda = 310$ – 350 nm leading to a regioselective addition of maleimide towards the end group carrying photoenol **12**. The system was subsequently irradiated for 4 h at $\lambda = 270$ – 310 nm forming **13**.

ability and robustness of the system. Thus, complex maleimide capped molecules carrying the fluorescent dye fluorescein^[15] or the antimicrobial peptide magainin were employed as reaction partners^[16] as well as species featuring electron-poor double bonds, such as acrylates and acrylic acid, in order to show the power of the ligation approach (see the Supporting Information).

A mixture of **9** with an equimolar amount of fluorescein maleimide **19** in dichloromethane was irradiated for 20 min with the PL-L lamp. One sample was subsequently mixed with an excess of acrylic acid and irradiated for 4 h with the Arimed B6 lamp. The molecules **9**, **22**, and **24** were analyzed by ESI-MS (Figure 4). The ESI-MS data unambiguously show that **9** was completely converted into **22** after the first irradiation sequence. The second irradiation step leads to a complete conversion into **24**.

Importantly, dilinker **9** was also shown to quantitatively react in a λ -orthogonal fashion with the fluorescein derivate **19** on both termini (Supporting Information, Figure S29) along with maleimide and methyl acrylate (Supporting Information, Figure S30) on the photoenol and tetrazole terminus.

Furthermore, on the basis of the small molecule studies, a λ -orthogonal polymer/peptide hybrid triblock synthesis starting from the α,ω -functional dilinker **9**, a maleimide terminal magainin peptide, and a maleimide terminal polylactide **16** (see the Supporting Information) was carried out. The light-induced λ -orthogonal click reaction sequence is illustrated in Scheme 2.

A mixture of **9** with an equimolar amount of magainin maleimide **21** in dry DMF was irradiated for 30 min in the wavelength range of $\lambda = 310$ – 350 nm (see the Supporting Information and Figure S31 for the MS analysis). Subse-

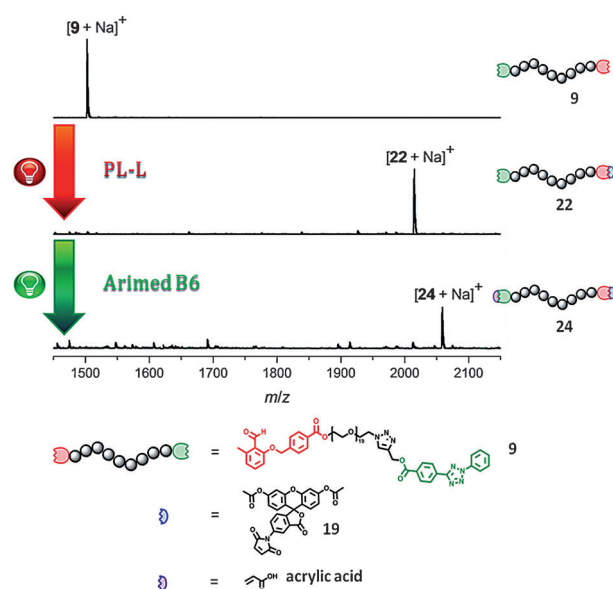
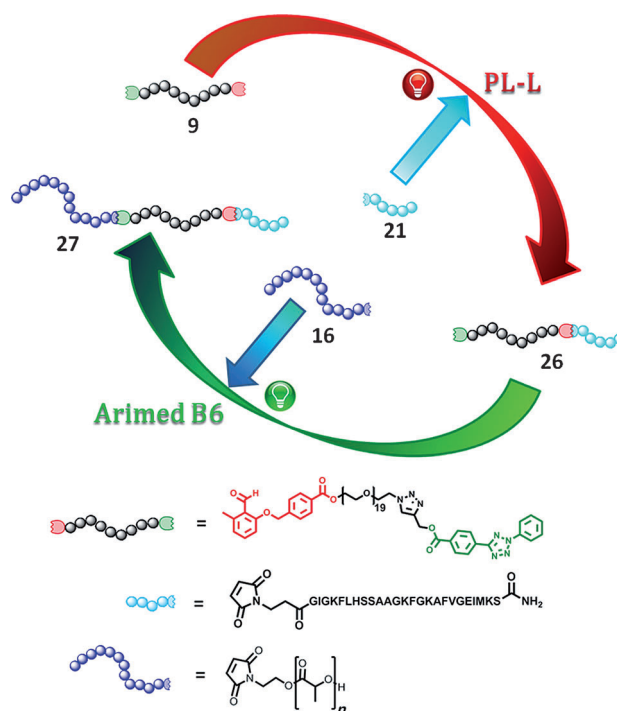


Figure 4. A system consisting of the dilinker **9** and fluorescein 5-maleimide **19** was irradiated for 20 min at $\lambda_{max} = 370$ nm leading to a regioselective addition of fluorescein towards the end group carrying the photoenol **22**. The system was subsequently irradiated for 4 h at $\lambda_{max} = 310$ nm forming **24** that carries an acid functionality at the tetrazole chain end.



Scheme 2. Stepwise construction of a triblock polymer/peptide hybrid starting from the α,ω -functional dilinker **9**. The irradiation with the PL-L lamp of **9** and an equimolar amount of the maleimide-capped magainin **21** leads to the site-specific formation of the magainin-PEG diblock **26**. The subsequent irradiation of the diblock with an equimolar amount of the maleimide terminal polylactide with the Arimed B6 lamp forms the hybrid triblock macromolecule **27**.

quently, the solution was mixed with a maleimide-capped polylactide ($M_n = 8000$ g mol⁻¹) and irradiated for 6 h ($\lambda =$

270–310 nm). The macromolecules **9**, **16**, **26**, and **27** were analyzed by GPC to confirm the λ -orthogonal formation of the hybrid triblock in two steps (Figure 5).

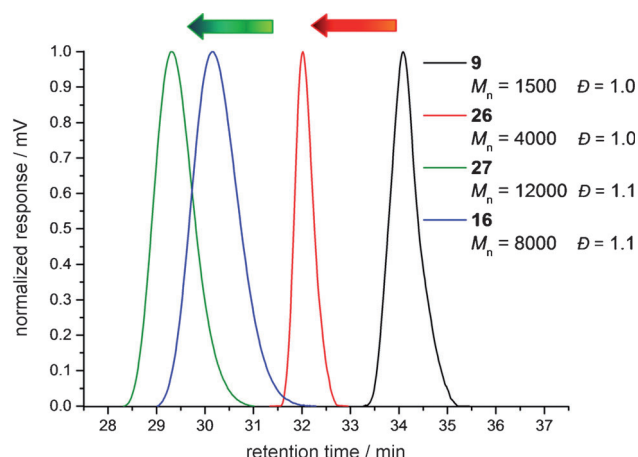


Figure 5. A system consisting of the dilinker **9** and an equimolar amount of magainin maleimide was irradiated for 30 min with the PL-L lamp selectively forming the peptide-PEG diblock **26**. The system was subsequently mixed with the maleimide capped polylactide **16** and irradiated for 6 h with the Arimed lamp forming the hybrid triblock **27**. GPC reported relative to a PMMA calibration.

The GPC data reveal a successful block formation, indicated by a clear shift of the maleimide capped polylactide peak after the irradiation with the PL-L lamp and a second shift after the irradiation with the Arimed B6 lamp. Furthermore, the λ -orthogonal block formation was carried out in an all-polymer system, that is, employing two polylactide blocks ($M_n = 4800 \text{ g mol}^{-1}$). Close inspection of the GPC trace of **14** (Supporting Information, Figure S22) indicates substructuring: A first shoulder is located at a retention time of 31 min and is associated with the residual equivalent of maleimide-capped polymer **11**. A second very small shoulder is visible at 34 min and belongs to a small amount of non-converted dilinker **9**. The irradiation of compound **14** at $\lambda_{\text{max}} = 310 \text{ nm}$ subsequently leads to the triblock polymer **15**. The λ -orthogonal triblock synthesis is additionally evidenced via ^1H NMR spectroscopy (Supporting Information, Figure S21). Similarly, the λ -orthogonal block formation functions cleanly with two polylactide blocks of 8000 g mol^{-1} (Supporting Information, Figure S23). Thus, each end of the dilinker **9** can be selectively addressed by a specific wavelength regime for the attachment of a macromolecular chain, be it a synthetic polymer or a peptide sequence.

In summary, we have demonstrated the high efficiency of λ -orthogonal pericyclic reactions on two polymers carrying a different photoactive endgroup each with maleimide in a one-pot reaction and on an α,ω -functionalized orthogonal dilinker that reacts selectively with maleimides. Importantly, we verify that complex maleimides can be site-selectively attached to the dilinker in a λ -orthogonal fashion, including those featuring biologically applicable functionalities (for example, peptides and fluorescent labels) as well as synthetic polymers. The tetrazole compound is able to react, along with

maleimides, with enes featuring electron-poor double bonds such as acrylates and acrylic acid, which extends the site-specific λ -orthogonality of the dilinker to also include a chemical orthogonality. These combined aspects constitute a chemical method that is easy to handle and very powerful for selectively addressing λ -orthogonal end groups. Thus, based on light-induced reactions performed at specific wavelengths, λ -orthogonal pericyclic photochemistry offers an efficient and fast avenue to synthesize complex (macro)molecules by addressing individual parts of a macromolecule with light of different wavelengths. We envisage that λ -orthogonal pericyclic reactions will not only find applications in complex syntheses, but also in fields such as functional photoresist design. Thus, λ -orthogonal pericyclic photochemistry combines the features of classic photochemistry (no catalyst required, no by-products formed, and thus no purification necessary) with light-induced orthogonality, making it a prime tool for site specific macromolecular ligation.

Received: November 5, 2014

Published online: January 23, 2015

Keywords: click reactions · orthogonal reactivity · photochemistry · photoenol · tetrazole

- [1] a) K. Matyjaszewski, J. Xia, *Chem. Rev.* **2001**, *101*, 2921; b) G. Moad, E. Rizzardo, S. H. Thang, *Aust. J. Chem.* **2005**, *58*, 379.
- [2] M. Allmeroth, D. Moderegger, D. Gündel, K. Koynov, H.-G. Buchholz, K. Rausch, F. Roesch, R. Zentel, O. Thews, *Biomacromolecules* **2013**, *14*, 3091.
- [3] C. N. K. Guimard, K. K. Oehlenschlaeger, J. Zhou, S. Hilf, F. G. Schmidt, C. Barner-Kowollik, *Macromol. Chem. Phys.* **2012**, *213*, 131–143.
- [4] N. M. C. Tria, K.-S. Liao, N. Alley, S. Curran, R. Advincula, *J. Mater. Chem.* **2011**, *21*, 10261.
- [5] K. Matyjaszewski, N. V. Tsarevsky, *Nature Chem.* **2009**, *1*, 276–288.
- [6] C. Barner-Kowollik, A. J. Inglis, *Macromol. Chem. Phys.* **2009**, *210*, 987.
- [7] a) H. C. Kolb, M. G. Finn, K. B. Sharpless, *Angew. Chem. Int. Ed.* **2001**, *40*, 2004; *Angew. Chem.* **2001**, *113*, 2056; b) J.-F. Lutz, *Angew. Chem. Int. Ed.* **2007**, *46*, 1018; *Angew. Chem.* **2007**, *119*, 1036; c) C. E. Hoyle, C. N. Bowman, *Angew. Chem. Int. Ed.* **2010**, *49*, 1540; *Angew. Chem.* **2010**, *122*, 1584.
- [8] E. P. Espeel, F. E. Du Prez, *Macromolecules* **2015**, *48*, 2–14.
- [9] a) B. Adzima, Y. J. Tao, C. Kloxin, C. A. J. DeForest, K. S. Anseth, C. N. Bowman, *Nat. Chem.* **2011**, *3*, 256; b) T. Pauloeherl, G. Delaitre, V. Winkler, A. Welle, M. Bruns, H. G. Börner, A. M. Greiner, M. Bastmeyer, C. Barner-Kowollik, *Angew. Chem. Int. Ed.* **2012**, *51*, 1071; *Angew. Chem.* **2012**, *124*, 1096; c) T. Pauloeherl, A. Welle, M. Bruns, K. Linkert, H. G. Börner, M. Bastmeyer, G. Delaitre, C. Barner-Kowollik, *Angew. Chem. Int. Ed.* **2013**, *52*, 9714; *Angew. Chem.* **2013**, *125*, 9896; d) M. A. Tasdelen, Y. Yagci, *Angew. Chem. Int. Ed.* **2013**, *52*, 5930; *Angew. Chem.* **2013**, *125*, 6044; e) A. Bogdanova, V. V. Popik, *J. Am. Chem. Soc.* **2003**, *125*, 14153; f) A. A. Poloukhine, N. E. Mbua, M. A. Wolfert, G. J. Boons, V. V. Popik, *J. Am. Chem. Soc.* **2009**, *131*, 15769.
- [10] N. J. Turro, V. Ramamurthy, J. S. Scaiano, *Modern Molecular Photochemistry of Organic Molecules*, University Science Books, University of California, **2010**.

- [11] V. San Miguel, C. G. Bochet, A. del Campo, *J. Am. Chem. Soc.* **2011**, *133*, 5380.
- [12] R. Franklin, H. Kim, S. A. Chambers, A. Scott, A. N. Mangham, R. J. Hamers, *Langmuir* **2012**, *28*, 12085.
- [13] H. Inui, S. Murata, *J. Am. Chem. Soc.* **2005**, *127*, 2628.
- [14] C. Barner-Kowollik, F. E. Du Prez, P. Espeel, C. J. Hawker, T. Junkers, H. Schlaad, W. Van Camp, *Angew. Chem. Int. Ed.* **2011**, *50*, 60; *Angew. Chem.* **2011**, *123*, 61.
- [15] a) N. Saruyama, Y. Sakukara, T. Asano, T. Nishiuchi, H. Sasamoto, H. Kodama, *Anal. Biochem.* **2013**, *441*, 58; b) C. P. Chan, Y. Bruemmel, M. Seydack, K. Sin, L. Wong, E. Merisko-Liversidge, D. Trau, R. Renneberg, *Anal. Chem.* **2004**, *76*, 3638.
- [16] a) L. Ryan, B. Lamarre, T. Diu, J. Ravi, P. J. Judge, A. Temple, M. Carr, E. Cerasoli, B. Su, H. F. Jenkinson, G. Martyna, J. Crain, A. Watts, M. G. Ryadnov, *J. Biol. Chem.* **2013**, *288*, 20162; b) O. Cirioni, C. Silvestri, R. Ghiselli, F. Orlando, A. Riva, F. Mocchegani, L. Chiodi, S. Castelletti, E. Gabrielli, V. Saba, G. Scalise, A. Giacometti, *J. Antimicrob. Chemother.* **2008**, *62*, 1332.