Endo and Exo Diels–Alder Adducts: Temperature-Tunable Building Blocks for Selective Chemical Functionalization

Emre H. Discekici, Andre H. St. Amant, Shay N. Nguyen, In-Hwan Lee, Craig J. Hawker, and Javier Read de Alaniz

Department of Chemistry and Biochemistry, ‡Materials Research Laboratory, and †Materials Department, University of California, Santa Barbara, California 93106, United States

ABSTRACT: The development and application of a novel endo furan-protected maleimide building block is reported. The endo isomer undergoes deprotection at temperatures ~50 °C below the exo derivative. This enables a simple and powerful approach to quantitatively and selectively introduce functional maleimide groups via temperature modulation.

The development of “click” chemistry has had a profound impact on applications ranging from small-molecule bioconjugation to the synthesis of complex and multifunctional macromolecular systems.1 Of the myriad of available “click” reactions, maleimides represent one of the most versatile building blocks, as they offer two distinct and highly efficient reaction pathways for secondary functionalization (see Figure 1). The first is a facile [4+2] Diels–Alder (DA) cycloaddition between electron-deficient maleimides and dienes. The second is a thiol-Michael reaction where a nucleophilic thiol adds across the maleimide double bond.2 Both pathways proceed quantitatively under equimolar conditions from a wide variety of starting materials. While the high reactivity of maleimides is desirable for post-polymerization functionalization, direct incorporation into polymers prepared through conventional free radical and controlled radical polymerization (CRP) is precluded.3−5 In previous work, Haddleton and co-workers developed an exo furan-protected maleimide atom transfer radical polymerization (ATRP) initiator for incorporation of a masked maleimide moiety at the α chain-end.6 The furan protecting group could then be removed via a retro-DA (rDA) process upon heating at elevated temperatures (~110 °C). Coupled with facile thiol-Michael addition, this strategy has significantly impacted the preparation of polymer–protein bioconjugates.7 Maynard and co-workers expanded this work, demonstrating successful incorporation of exo furan-protected maleimides in reversible addition–fragmentation chain-transfer (RAFT) polymerization processes.8 Dove and Sanyal also demonstrated successful utility in ring-opening polymerization (ROP) systems.9,10 Despite the importance of maleimide incorporation to the field of functional polymer synthesis, only the exo isomer has been explored as a functionalization platform. As such, the inherently high deprotection temperature is problematic for thermally unstable systems, such as bioconjugates and supramolecular assemblies.2,11 To address this challenge, we hypothesized that the endo isomer, which undergoes rDA at considerably lower temperatures12,13 would afford a new functional building block with the added benefit of temperature tunability.

Our initial exploration focused on a scalable and straightforward synthesis of the endo adduct, I. Using inexpensive and readily available starting materials (furan and maleimide), a mixture of DA adducts enriched with I can be obtained when the cycloaddition reaction is performed at room temperature. From this mixture, the endo isomer, I, is selectively recrystallized from dichloromethane on up to a 4-gram scale (23% yield, see Supporting Information (SI)). The reaction of I with the exo isomer, 2,14 results in the endo/exo heterodimer model compound (3), which offers the possibility of selective deprotection and separate/successive thiol-Michael addition steps. Significantly, when 3 was heated at 60 °C, only deprotection of the endo adduct was observed (see SI). While extended reaction time for complete deprotection is necessary, nucleophilic addition with n-dodecanethiol enables exclusive monofunctionalization of the heterodimer to give 3-mono in good yield (Figure 2). Subsequent deprotection of the exo...
adduct at 100 °C followed by reaction with 4-trifluorobenzyl mercaptan furnished the final disubstituted compound 3-di (Figure 2), confirmed by 1H, 13C, and 19F NMR and electrospray ionization mass spectrometry (ESI-MS) analysis (Figures S18−S20).

With selective conjugation achieved on a small-molecule heterodimer, our efforts focused on adapting this chemistry to facilitate orthogonal post-functionalization of synthetic polymers. To obtain the necessary polymeric building blocks, a key hydroxyethyl precursor (4) was synthesized in one step from 1 (Scheme 1) with single-crystal X-ray analysis confirming the endo conformation of 4 (see SI). A traditional ATRP initiator (5a) and methacrylate monomer (6a) bearing the endo isomer could then be obtained from 4 using 2-bromoisobutyryl bromide and methacryloyl chloride, respectively.

Given the lower deprotection temperature of the endo isomer and the associated incompatibility with traditional thermally driven radical polymerization techniques, our attention was drawn to ATRP systems that operate under ambient temperatures. Initial investigation of the viability of 5a for Cu(0) polymerization15 reveals a bimodal distribution at moderate to high conversions (Figure S23). This is in agreement with previous reports that suggest protected maleimides can still participate in copolymerizations6 when using Cu-ATRP. To address this issue, we turned to light-mediated CRP techniques, namely metal-free ATRP16−20 and photoinduced electron transfer-RAFT (PET-RAFT),21−23 for direct incorporation of temperature sensitive functionalities into polymeric scaffolds. Implementation of metal-free ATRP with purified endo- and exo-monomers would therefore allow the development of multifunctional polymers that leverage the selective deprotection temperatures of the endo and exo building blocks (Figure 1a), opening up the range of functionalization chemistries available in synthetic polymer systems. Significantly, metal-free ATRP using Phen-CF3 yielded a unimodal distribution with low D̅ for the endo-initiator (Figure 3), while PhenO (see SI) allows 6a (endo adduct) to be successfully copolymerized with 6b (exo adduct), methyl methacrylate (MMA) and benzyl methacrylate (BnMA). The choice of photocatalyst was based on

Scheme 1. Synthesis of Endo/Exo Heterodimer (3) and Endo Polymer Building Blocks (5a, Initiator; 6a, Monomer) a

Reagents and conditions: (i) K2CO3, MeCN, RT, 56%; (ii) 2-bromoethanol, K2CO3, MeCN, RT, 73%; (iii) BIBB, Et3N, DCM, 0 °C to RT, 84%; (iv) methacryloyl chloride, Et3N, DCM, 0 °C to RT, 80%. For exo polymer building blocks (5b, initiator; 6b, monomer) see SI.

Figure 2. (a) Scheme and (b) 1H NMR overlay of orthogonal deprotection and thiol-maleimide coupling reactions using small-molecule endo/exo heterodimer, 3. Reagents and conditions: (i) DMP, 60 °C, 22 h; (ii) n-C6H5SH, TEA, CHCl3, RT, 15 h, column chromatography, (93%, two steps); (iii) toluene, 100 °C, 17 h, >95% conversion of exo; (iv) p-CF3BnSH, TEA, CHCl3, RT, 4 h, column chromatography (81%, two steps).

Figure 3. (a) Scheme of metal-free ATRP of MMA using the endo ATRP initiator, 5a. (b) Size exclusion chromatography confirming unimodal distribution with low D̅.
optimal compatibility with the initiator-type as demonstrated in previous reports.\textsuperscript{19,20} This represents the first copolymer (P2) to contain both endo and exo isomers (Figure 4a). The addition of BnMA serves as a covalently bound internal \( ^1\)H NMR reference to facilitate reliable determination of the efficiency of selective deprotection. One of the most attractive features of a CRP is the ability to impart site-specific control over a desired functionality. Heating the copolymer in DMF-d\(_6\) at 60 °C results in complete deprotection of the endo isomer (Figures 4b and S27). \textit{In situ} addition of 4-trifluoromethyl benzyl mercaptan and characterization by \( ^1\)F NMR confirmed the fidelity of deprotection to maleimide and the associated reactivity toward thiols (Figures S27–S30). Importantly, subsequent heating to 110 °C after endo functionalization resulted in quantitative deprotection of the remaining exo isomer to furnish the reactive maleimide (Figures S31 and S33).

We then envisioned the preparation of a multifunctional copolymer, wherein one isomer is incorporated on the chain-end and the other as a pendant group along the backbone. Using 5a, MMA was successfully copolymerized with 6b to furnish a functional copolymer (P5) with good control (Figure S34). Having successfully demonstrated thiol-Michael addition after selective deprotection of the endo isomer, we performed selective deprotection at the \( \alpha \) terminus and trapped the \textit{in situ} generated maleimide with an irreversible DA cycloaddition with cyclopentadiene (Cp) end-capped poly(ethylene glycol) (P4). Inspired by recent reports from Barner-Kowollik and co-workers, Cp end-capped polymers represent a powerful strategy for highly efficient polymer functionalization.\textsuperscript{24–26} Indeed when P4 (see SI) and P5 were heated together in solution, a facile and catalyst-free preparation of diblock copolymer (P6), with retention of the exo functionality, was achieved (Figure S5a,b). While exo protected maleimides can undergo deprotection to reveal a reactive maleimide, they can also be used for ring-opening metathesis polymerization (ROMP),\textsuperscript{27} radical thiol–ene “click”,\textsuperscript{28} and inverse electron-demand DA (IEDDA) reactions.\textsuperscript{29} The IEDDA reaction with tetrazines has found widespread use in polymer conjugation and chemical biology due to the ability to achieve bioorthogonal, catalyst-free conjugation under mild conditions.\textsuperscript{30–32} Significantly, in a one-pot fashion, 3,6-bis(methoxy carbonyl)-1,2,4,5-tetrazine was added to P6, resulting in quantitative consumption of the remaining exo isomer as evidenced by \( ^1\)H NMR and size exclusion chromatography (SEC)–UV analysis (Figure S5b,d). Importantly, in a similar fashion to the dual functionalization of the small-molecule heterodimer (3), P5 can be heated to 60 °C in the presence of \( n \)-dodecanethiol (3), resulting in quantitative deprotection of the exo-isomer and the resulting maleimide was reacted with 4-trifluorobenzyl mercaptan in a one-pot fashion to yield the dual thiol-Michael addition product (Figures S39 and S40). Analysis of the starting polymer and the final dual addition polymer by SEC-RRI indicates no observable change of the molar mass distribution or overall dispersity (Figure S41). Furthermore, the versatility of this method also enables synthetic access to the inverse orientation of P5, with the exo isomer on the chain-end and the endo as pendant groups (P8, Figure S42).

In conclusion, we have developed a straightforward and scalable synthesis for an endo furan-protected maleimide functional building block and demonstrated its facile incorporation into two distinct systems: a difunctional small-molecule endo/exo heterodimer and a multifunctional synthetic polymer with control over chain ends and pendant backbone groups. By implementing metal-free ATRP and co-incorporating the exo isomer, we highlight key advantages of using mild CRP for the design of materials with tunable functionalities that undergo selective deprotection based on temperature. Furthermore, we have demonstrated the utility of this chemistry through a series of site-specific and quantitative modifications using established and commonly implemented “click” reactions, including thiol-Michael addition, DA, and IEDDA conjugation chemistries. We envision that the ability to selectively introduce functionality based on external temperature regulation will pave new pathways forward for efficient and precision small-molecule and polymer modification. Further development of different functional Diels–Alder derivatives and investigation into additional synthetic polymer applications is currently underway.

![Figure 4](image-url)

*Figure 4.* (a) Copolymer P2 (synthesized using 6a, 6b, MMA, and BnMA, with PhenO and 380 nm light) is heated to selectively deprotect the endo isomer to yield P3 (see SI for synthetic details). (b) Plot depicting stability of the exo isomer and quantitative deprotection of the endo isomer at 60 °C over 16 h.
Figure 5. (a) Representative schematic of one-pot selective endo deprotection and DA cycloaddition conjugation with PEG-Cp followed by functionalization of remaining pendant exo functionality through IEDDA with 3,6-bis(methoxycarbonyl)-1,2,4,5-tetrazine. (b) Crude $^1$H NMR overlay confirming successful conversion after each reaction. (c) SEC-RF overlay showing shift to higher molar mass following diblock formation after heating P4 and P5 at 60 °C for 18 h. (d) SEC-UV @275 nm overlay of P6 and P7 confirming conjugation with bis(methoxycarbonyl)-1,2,4,5-tetrazine after 1 h at room temperature.

**REFERENCES**

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