Click Conjugations

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Ultrafast Click Conjugation of Macromolecular Building Blocks at Ambient Temperature**

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The combination of highly efficient orthogonal conjugation chemistries with controlled free-radical polymerization has increasingly proven to be a convenient tool in the synthesis of novel polymeric materials.[1] For example, techniques such as atom-transfer radical polymerization (ATRP) and reversible addition–fragmentation chain-transfer (RAFT) polymerization have successfully been combined with click chemistry to achieve a wide variety of structures ranging from complex architectures (e.g. blocks,[2–4] stars,[5–7] and combs[8,9]) to conjugates of synthetic polymers and biomolecules such as peptides and proteins,[10–12] sugars,[13,14] and even viruses.[15] The continued development of such techniques is ultimately geared towards faster, more efficient reactions that may be performed under ambient conditions and utilizing benign or, indeed, no catalysts.

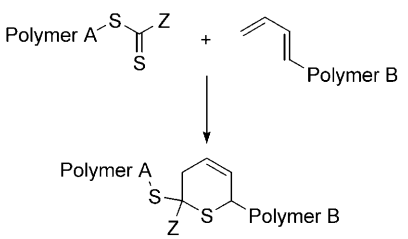
Within the realm of polymer chemistry, the copper(I) azide–alkyne cycloaddition (CuAAC) has been the most widely utilized click reaction, owing to its high selectivity and efficiency under relatively mild reaction conditions. One of the major limiting factors of the CuAAC is the requirement of a toxic copper catalyst. This drawback can have a profound influence on its compatibility with many systems that are sensitive to heavy metals, particularly in biological applications.[16] Although there are examples of "copper-free" azide–alkyne cycloadditions,[17,18] they have not obtained the overall popularity of the CuAAC.

In an attempt to address some of the issues surrounding the applicability of click chemistry, a number of alternative strategies have been proposed. For instance, the Diels–Alder cycloaddition between anthracene and maleimide derivatives has proven to produce excellent results in the formation of complex architectures in a modular approach.[4,7] However, a severe drawback to this technique is the requirement of temperatures in excess of 110 °C and long reaction times (36–120 h). Thus, this technique would not be appropriate for use in conjugation reactions involving proteins and nucleic acids, which can readily denature under such conditions.

The CuAAC reaction generally requires reaction times of several hours at temperatures ranging from ambient to 50 °C. However, in the synthesis of star polymers by a click coupling method, Gao and Matyjaszewski report a 97 % conversion of all azide moieties into 1,2,3-triazole groups within 3 h at room temperature (using 1:1 stoichiometry).[5] Moreover, van Camp et al. report the completion of a click reaction between azide-functionalized poly(isobornyl acrylate) and two equivalents alkyne-functionalized poly(1-ethoxyethyl acrylate) in just five minutes under ambient conditions.[9] It is therefore apparent that the rate of the CuAAC can be influenced by using an excess of one of the reactants; however, this approach is undesirable in the majority of cases, as further purification strategies are necessary. Furthermore, there is a growing attraction in the polymer community to thiol–ene chemistry, which, under certain conditions, can be completed in 5 min to 2 h.[19,20]

Recently, we reported several examples of the highly atom-economical RAFT–HDA concept in the efficient construction of block copolymers,[21] stars,[22,23] and surface-functionalized microspheres.[24] In these examples, polymers prepared by RAFT polymerization in the presence of electron-deficient dithioesters have been conjugated to materials bearing a suitable diene through a hetero-Diels–Alder (HDA) cycloaddition (Scheme 1). To date, the reactions used have been performed at 50 °C, have taken between 2 and 24 h to achieve completion, and have made use of *trans*,*trans*-2,4-hexadien-1-ol as the diene.

Herein, we demonstrate a dramatic reaction-rate improvement of the RAFT–HDA click reaction through the use of novel cyclopentadienyl-functionalized polymers. In analogy to approaches widely utilized in the synthesis of



Scheme 1. The RAFT–HDA concept. Z = electron-withdrawing group.

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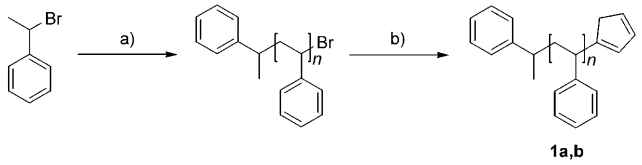
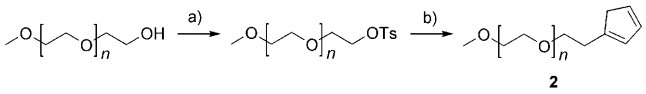
cyclopentadienyl ligands[25] and alkylated cyclopentadiene compounds,[26,27] polystyrene prepared by ATRP and bearing a terminal bromine substituent was treated with sodium cyclopentadienide in THF to achieve a complete substitution of the bromine atom with a cyclopentadienyl moiety (Scheme 2), as evidenced by ^{1}H NMR spectroscopy (see



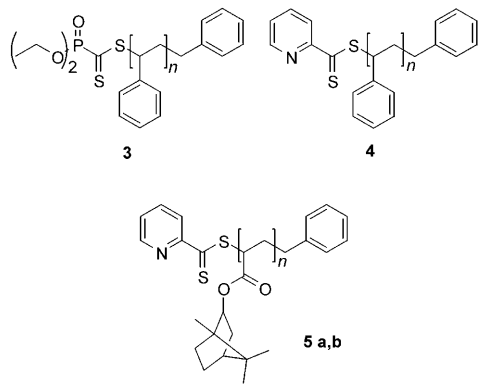
Scheme 2. Synthesis of cyclopentadienyl-terminated polystyrene by ATRP. a) ATRP of styrene, Cu^{I}Br/PMDETA, 90 °C; b) NaCp (2.0 M in THF), 0 °C–RT. PMDETA = N,N,N′,N″,N″-pentamethyldiethylenetriamine. **1a** and **1b** represent poly(styrene)s of different molecular weights.

Figure S5 in the Supporting Information). Concomitantly, commercially available poly(ethylene glycol) monomethyl ether was also equipped with a cyclopentadienyl end group through nucleophilic substitution of a tosylated intermediate (Scheme 3).



Scheme 3. Synthesis of cyclopentadienyl-terminated poly(ethylene glycol) (PEG). a) TsCl, pyridine, RT; b) NaCp (2.0 M in THF), THF, 0 °C–RT. Ts = Tosyl.

As dienophiles in the RAFT–HDA concept, benzyl(diethoxyphosphoryl)dithioformate was used to prepare polystyrene (PS) **3**, and benzylpyridin-2-yldithioformate was used to prepare polystyrene **4** and poly(isobornyl acrylate) **5a,b** by RAFT polymerization (Scheme 4). The molecular weight assessment of these building blocks is presented in Table 1.



Scheme 4. Polymers prepared by RAFT polymerization serving as dienophiles in the RAFT–HDA click concept. **5a** and **5b** represent poly(isobornyl acrylate)s of different molecular weights.

Table 1: Polymer characterization.[a]

Polymer	$M_{n,theo}$[b] [g mol^{-1}]	$M_{n,GPC}$ [g mol^{-1}]	$M_{n,NMR}$ [g mol^{-1}]	PDI[c]
1a	–	1830	1750	1.10
1b	–	3470	3350	1.10
2	–	2770	2050	1.04
3	–	2500	3080	1.11
4	–	3250	3340	1.12
5a	–	3380[d]	3510	1.15
5b	–	6620[d]	6950	1.14
1a-*b*-**3**	4830	4750	–	1.15
1a-*b*-**4**	5090	5420	–	1.12
1b-*b*-**4**	6810	6930	–	1.11
1b-*b*-**5a**	6460	7040	–	1.17
1b-*b*-**5b**	10300	10340	–	1.15
2-*b*-**3**	5130	5460	–	1.10
2-*b*-**4**	5390	5950	–	1.13
2-*b*-**5a**	5560	6110	–	1.15

[a] All reactions resulting in block copolymers were performed in chloroform at ambient temperature and pressure and were complete within 10 min. [b] Calculated from the sum of the individual blocks. [c] Polydispersity index. [d] Values for P*i*BoA have been corrected by applying the Mark-Houwink-Sakurada relationship against poly(methyl methacrylate) standards (K = 1.141 dL g^{-1}, α = 0.994).

The corresponding molecular weights as determined by NMR spectroscopic analysis are in good agreement with the data from gel permeation chromatography (GPC). The molecular weights derived from NMR spectroscopy were used for all calculations.

As an initial validation of our approach to achieve ultrafast click couplings and to ascertain the required reaction conditions, a series of simple model reactions was performed in which PS **3** and **4** were reacted with PS **1a** and **1b** in chloroform solution at room temperature. In the case of PS **4**, trifluoroacetic acid (TFA, 1.5 equiv) was added to catalyze the reaction. After shaking for 10 min, the solvent was removed in vacuo and the residue directly analyzed by GPC. The analysis shows a clear increase in molecular weight, in excellent agreement with the predicted value (as determined by the sum of the molecular weights of the individual blocks). This methodology was then utilized for all subsequent couplings.

Figure 1 shows an overlay of GPC traces of the individual polymeric building blocks and of the room-temperature stable coupling product for two selected examples (Scheme 5). In both cases, a clear shift of the trace to lower retention times indicates the successful block formation, which is consistent with the data presented in Table 1.

In previous publications concerning the use of polymers similar in structure to **3**,[21,22,24] zinc chloride has been required to catalyze the HDA cycloaddition. Herein, however, it was found that the coupling reaction proceeded to completion within 10 min without the addition of catalyst. This difference is attributed to the high Diels–Alder activity of the cyclopentadienyl end group. Furthermore, the high efficiency of the performed conjugations was demonstrated by deconvolution of the GPC data, which are presented in the Supporting Information.

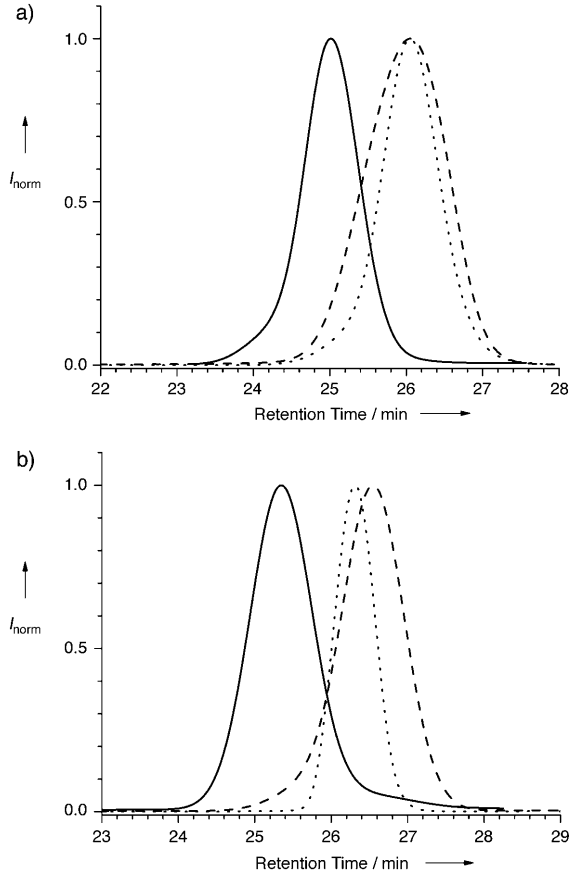
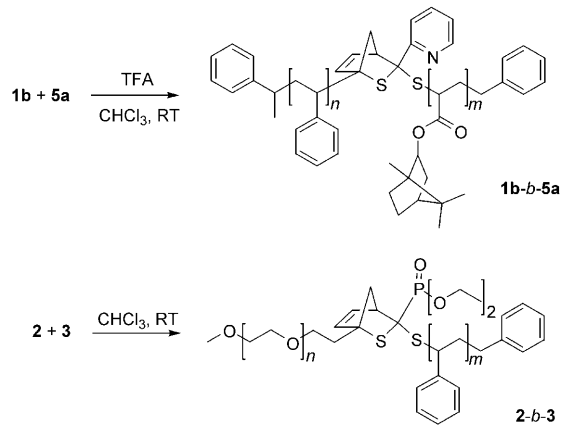


Figure 1. Overlay of GPC traces showing the formation of a) PS-*b*-P*i*BoA **1b**-*b*-**5a** (——) from PS **1b** (•••••) and P*i*BoA **5a** (-----); and b) PEG-*b*-PS **2**-*b*-**3** (——) from PEG **2** (•••••) and PS **3** (-----).



Scheme 5. Selected examples of the formation of block copolymers by ultrafast HDA chemistry. a) PS-*b*-P*i*BoA **1b**-*b*-**5a**, b) PEG-*b*-PS **2**-*b*-**3**. P*i*BoA = poly(isobornyl acrylate).

To assess the rate of the HDA coupling in a more quantitative fashion by GPC analysis, it is necessary to enforce a cessation of the HDA reaction at specific time intervals. We have previously reported that the pyridinyl dithioester is a more efficient heterodienophile than the phosphoryl dithioester[22] thus it was with polymers bearing the former end group that we investigated the kinetics of the rapid cycloaddition. Unlike the phosphoryl dithioester end group, which is permanently electron-withdrawing, the pyridinyl dithioester end group must be activated by protonation to undergo rapid HDA chemistry. Therefore, the pyridinyl dithioester serves as a molecular switch, which offers great control over the conjugation reaction. This property was utilized advantageously in determining the rate of block formation.

In a control run, PS **4** and PS **1a** were dissolved in chloroform without the addition of TFA. The mixture was allowed to stand at room temperature for one hour, after which the solvent was removed in vacuo and the residue directly analyzed by GPC. The resulting GPC trace was identical to that of a freshly prepared mixture of PS **4** and PS **1a** and consistent with the traces of the individual segments. It has been documented that pyridinyl dithioesters can react with butadiene derivatives without catalysis;[28] however, the reaction is so slow that it may be neglected herein. A stock solution of PS **4** and PS **1a** in chloroform was then prepared and distributed among four vials. The progress of the rapid HDA cycloaddition was then monitored by stopping the reaction and analyzing the crude reaction mixture by GPC. Reaction cessation was achieved by direct precipitation, after the allotted time, in cold basic methanol, which served to neutralize the acidic catalyst and recover the formed block copolymer. Figure 2 shows an overlay of the GPC traces of

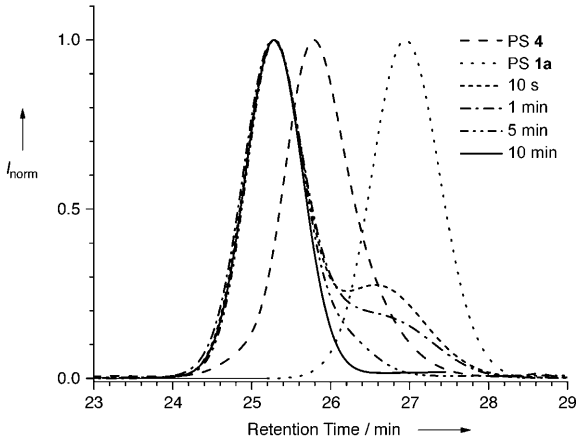


Figure 2. Overlay of GPC traces showing the progress of the HDA reaction between PS **4** and PS **1a**.

the starting materials PS **4** and PS **1a** along with those of the crude reaction mixtures after 10 s and 1, 5, and 10 min. Inspection of Figure 2 clearly demonstrates that the majority of the block copolymer structure is formed within the first 10 s of the reaction, and quantitative conversion is achieved within 10 min.

In summary, we have presented an efficient and extremely rapid room-temperature conjugation strategy to access pure block copolymer structures that proceeds without the addition of a catalyst. Furthermore, we have developed a technique to prepare novel cyclopentadienyl-functionalized polymers, which are easily accessible by ATRP. Thus, the click

concept presented herein allows the ultrafast conjugation of virtually all polymer strands accessible by RAFT and ATRP.

Experimental Section

All experimental procedures, NMR spectra, and GPC traces of all click couplings conducted in this investigation are outlined in the Supporting Information.

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