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Additive-Free Clicking for Polymer Functionalization and Coupling by Tetrazine–Norbornene Chemistry

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Supporting Information

ABSTRACT: Herein we report the use of a tetrazine-norbornene inverse electron demand Diels–Alder conjugation applied to polymer end-functionalization and polymer–polymer coupling. The reaction was found to be applicable to polymer–polymer coupling, as judged by SEC, DOSY NMR, and LCxSEC analyses, giving diblock copolymers by merely mixing the constituent homopolymers together under ambient conditions, using no catalyst, additive, or external stimulus.

The applications of the wide array of 'click' reactions are seemingly limitless and are found throughout the chemical, biological, and materials fields. Since its introduction in 2001 by Sharpless and co-workers[1] the concept has found wide-ranging applications; however, its use in polymer science,[2] where the efficiency of these approaches proffers an enhanced ability to readily modify polymer chain ends or carry out polymer–polymer conjugation,[3] has led to the click philosophy and family of reactions being widely embraced.

Arguably, the copper-catalyzed azide–alkyne Huisgen 1,3-dipolar cycloaddition (CuAAC)[4] has become the *de facto* standard for many applications where click reactions are required and thus has become almost synonymous with the term 'click reaction'. Although it undoubtedly fulfils all of the requirements of a click reaction, the requirement for a metal catalyst and its subsequent complete removal can be a limitation, particularly for biomedical applications, as well as a concern over the stability/explosive nature of low-molecular weight azido species. There is also no single Cu(I) complex that has been identified as a suitable 'off the shelf' catalyst or precatalyst for CuAAC under all conditions, thus requiring tailoring of conditions for each reaction, particularly in organic media.

Many metal-free click reactions have been reported,[5] including the (hetero-)Diels–Alder and several 'thiol–ene' reactions.[6] However, each reaction has limitations;[7] for instance, radical-based 'thiol–ene' reactions are insufficiently efficient to enable polymer–polymer coupling[8] and cannot be applied to radical-sensitive reactions such as DNA ligation. In a wider sense, thiol-containing compounds also suffer from significant disulfide formation, especially in oxophilic aqueous solvents, while Michael-addition reactions using thiols generally need catalysts/initiators to proceed at acceptable rates.[9] Likewise, the reversibility of Diels–Alder reactions[10] can act as both a blessing and a curse, in that it can be useful for some applications,[11] but in others instability of the linkage can be undesirable.

Scheme 1. Tetrazine–Norbornene DA_{inv} Ligation[a]

[a] For a fuller ligation mechanism, see ref 12 or Scheme S1 in SI.

The inverse electron demand Diels–Alder (DA_{inv}) reaction between tetrazines and strained alkenes or acetylenes to yield dihydropyridazines or pyradizines (Scheme 1) has attracted much less attention. Nonetheless, it is fast, atom efficient, catalyst free, air insensitive, and quantitative and hence fulfils all of the requirements of the click concept.[12] Although first discovered over 50 years ago,[13] it has since been employed in bioconjugation,[14] conjugation to quantum dots,[15] and in the modification of DNA.[16] In recent highlights[7,17] however, it was the only example from an extensive list of reactions denoted as 'click' that had not yet been applied to polymer synthesis. Thus, the DA_{inv} reaction is herein demonstrated to provide an outstanding method for the facile ligation and modification of polymers, including polymer–polymer couplings in a range of solvents.

To demonstrate the potential utility of the tetrazine–norbornene click reaction in polymer functionalization and coupling, we first explored the orthogonality of this reaction[18] with respect to commonly utilized initiator functionalities and catalysts in controlled polymer synthesis, particularly the thiocarbonyl group found in reversible addition–fragmentation chain transfer (RAFT) agents. RAFT chemistry is a highly versatile method for the synthesis of a wide range of functional and responsive polymers,[19] which imparts a sensitive thiocarbonyl functionality at a chain end that can be directed to undergo a wide variety of postpolymerization modifications.[20] Therefore, the orthogonality of the DA_{inv} reaction with respect to the trithiocarbonate functionality of a previously reported norbornene (Nb)-functionalized chain transfer agent (CTA) Nb-TTC[21] (Schemes S3 and S6 in SI) was initially investigated to confirm the retention of the trithiocarbonate functionality

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throughout the reaction as well as provide a model system to establish the scope of the reaction with regard to solvent choice. Nb was chosen as the dienophile as a consequence of its excellent activity in the DA_{inv} reaction[22] and also as it is a commonly used motif in the polymer community for ring-opening metathesis polymerization (ROMP).[23]

Addition of dipyridyltetrazine Tz(pyr)$_2$, which is known to have markedly increased reactivity when compared to that of the corresponding diphenyltetrazine,[13,14d] to Nb-TTC in equimolar quantities in CH_2Cl_2 solution demonstrated the orthogonality of this coupling reaction to the RAFT end group. Observation of the predicted product by mass spectrometry and the expected disappearance of the signal from the Nb double bond in the CTA from the ^{1}H NMR spectrum (~6.1 ppm), while retaining the methylene signals from protons adjacent to the trithiocarbonate group at the same resonances confirmed that the reaction proceeded cleanly (see Figure S7 in SI). Although the ^{1}H NMR spectrum of the coupled product is complicated by the different stereo- and regioisomers formed upon ligation, it was possible to isolate the oxidized product by column chromatography and fully assign all peaks in the coupled product. Solvent screening on the model system was also carried out to determine the range of solvents in which the reaction can be performed as well as their effect on the reaction rate. Dichloromethane, ethanol, dimethylsulfoxide, tetrahydrofuran, ethyl acetate, *N*,*N*-dimethylformamide, and 1,4-dioxane were tested in duplicate by sampling the reaction mixture after 1 or 2 h, respectively, and analyzing the reaction mixture by LC−MS. The rate was found to be affected by the solvent according to the following order: DMSO > DMF ≈ EtOH > 1,4-dioxane ≈ THF ≈ CH_2Cl_2, as measured by the relative integrations of the Tz(pyr)$_2$ peak. As expected, three conjugation adducts were observed due to the aforementioned isomerism, with the ratios of isomers dependent on solvent choice (see Figure S9 in SI). This highlights the scope of this click reaction toward polymer−polymer couplings where good solubility of the polymers is often a key consideration.

To explore the rate of the tetrazine−norbornene reaction, *in situ* monitoring by ^{1}H NMR and UV/vis spectroscopy was carried out on the model system in CH_2Cl_2. All experiments were performed at ambient temperature in air, using standard grade solvents. At a concentration of 0.06 M (with equimolar starting materials), the reaction was complete within 50 min, with no starting materials detectable by ^{1}H NMR spectroscopy (see Figure S8 in SI). The coupling reaction progress is also characterized by a distinctive color change that can be monitored by UV/vis spectroscopy utilizing the weak absorbance at ~546 nm. Varying reaction concentrations (0.06−0.001 M) and equivalents of Tz(pyr)$_2$ (1−10 equiv) demonstrated that, as would be expected, the rate of coupling increased with higher concentration and equivalents of Tz(pyr)$_2$ with respect to the Nb group (Figure S10 in SI).

Norbornenyl-functionalized poly(styrene) (PS-Nb, **1**, **2**) and poly(*N*-isopropylacrylamide) (PNIPAM-Nb, **3**) were synthesized *via* RAFT polymerization using the above-mentioned Nb-CTA Nb-TTC,[21] in an analogous manner to that described previously by the Wooley[24] and Advincula groups.[25] The molecular weight was determined by SEC, and the degree of retained norbornenyl functionality was confirmed by comparison of the integration of the Nb signal at 6.1 ppm with the expected integration of the aromatic styrene or amide (~6.3−7.2 ppm) signals in the ^{1}H NMR spectrum. As a consequence of high polymerization conversions leading to lower end group fidelities,

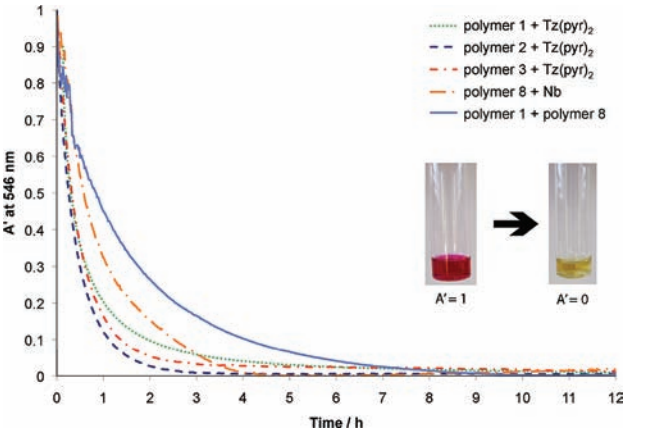

Figure 1. UV/vis trace showing the progress of the polymer−small molecule and polymer−polymer click reactions in CH_2Cl_2 in equimolar ratios. A' = normalized UV absorbance at 546 nm.

conversions were kept low (~25%) to give Nb functionalization of up to 95%. Poly(ε-caprolactone) (PCL-Nb, **4**) and poly(δ-valerolactone) (PVL-Nb, **5**−**7**) were accessed *via* ring-opening polymerization (ROP) using 5-norbornene methyl alcohol as an initiator.[26]

A postpolymerization modification was carried out to access a tetrazine-functionalized PEG by coupling a commercially available PEG with an acid-functionalized tetrazine, which was synthesized according to a literature precedent (Scheme S4 in SI).[27] Unfortunately, the reactivity of tetrazine toward vinyl-based monomers[28] means that it is unsuitable for incorporation into a RAFT, NMP, or ATRP initiator. However, we were able to directly synthesize PVL-Tz **9** *via* ROP using an alcohol-functionalized tetrazine[29] as an initiator.

Since not only polymer−polymer coupling but also the fast and quantitative end-modification of polymers is a desirable target, the same conditions as in the small-molecule study were utilized to functionalize three different polymers **1**, **2**, and **3**. Little to no difference was found in the reaction rate between the small-molecule model reaction, described above, and the reaction rate for polymer−small-molecule coupling, or between polymers of different types and molecular weights (see Figure 1). Indeed, even for the functionalization of a 16.4 kDa PNIPAM, the equimolar coupling reaction reached over 90% conversion in just 3 h.

Confirmation of completion of the coupling reaction is made simple as a result of distinct signals in the ^{1}H NMR spectrum in regions that generally contain few other signals. Most notably, the norbornenyl resonance at 6.1 ppm disappears completely, and new signals between 7.6−9.3 ppm, which can be readily assigned to the clicked tetrazine end groups, appear concomitantly (Figure 2). End group modification of a higher-molecular weight PS-Nb, **2**, (14.5 kDa) showed the same pattern of signals in the ^{1}H NMR spectrum, as did the water-soluble PNIPAM-Nb, **3** (16.4 kDa) (Table 1 and Figure S12 in SI).

To further explore the potential scope of the reaction, end-functionalization of PEG-Tz **8** with a water-soluble Nb-containing compound (5-norbornene-2-*endo*,3-*endo*-dimethanol) was carried out in water. The reaction proceeded at a very similar rate when compared to that of the functionalization of PS and PNIPAM, and efficiency was determined by ^{1}H NMR and MALDI mass spectrometries (Figures S13, S14 in SI). Due to the propensity of the tetrazine group to coordinate metals,[30] it

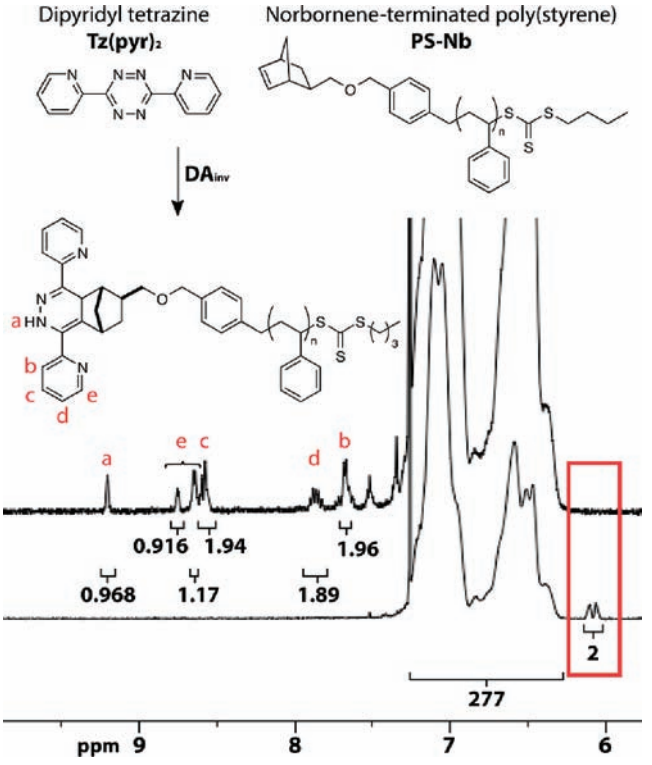

Figure 2. Partial ^{1}H NMR spectrum of PS-Nb **1** pre- (*bottom*) and post- (*top*) click with Tz(pyr)$_2$ (400 MHz, $CDCl_3$). Quantitative functionalization is shown by the disappearance of norbornenyl 2H (red box, 6.1 ppm), and appearance of 9H (a−e) from the clicked tetrazine.

Table 1. Characterization Data for the Polymers Used in This Study

entry	polymer	M_n /kDa[a]	M_w/M_n[b]
1	PS-Nb	5.6	1.43
2	PS-Nb	14.5	1.25
3	PNIPAM-Nb	16.4	1.21[c]
4	PCL-Nb	5.6	1.05
5	PVL-Nb	4.9	1.08
6	PVL-Nb	10.7	1.07
7	PVL-Nb	31.0	1.08
8	PEG-Tz	5.4	1.04
9	PVL-Tz	1.7	1.23

[a] Calculated by ^{1}H NMR. [b] Calculated by SEC (THF, PS standards). [c] Calculated by SEC (DMF, PMMA standards).

proved difficult to obtain a MALDI spectrum of **8**; hence, conjugation was confirmed by means of calculating the mass shift with reference to the starting PEG-amine. The isolated polymer was found to be fully oxidized, as evidenced by the lack of characteristic signal at ~9.2 ppm in the ^{1}H NMR spectrum.

The same reaction protocol (0.01 M in CH_2Cl_2, room temperature) was carried out for conjugation of PEG-Tz **8** and PS-Nb **1** to afford an amphiphilic block copolymer (Figure 3). Given the results of the solvent screening, we also performed the reaction in a 1:1 CH_2Cl_2/DMSO mixture to expedite the reaction. In both cases, the resulting SEC traces were identical, although the reaction proceeded faster in the mixed solvent than in pure CH_2Cl_2. Although not 'ultrafast',[31] the reaction proceeds

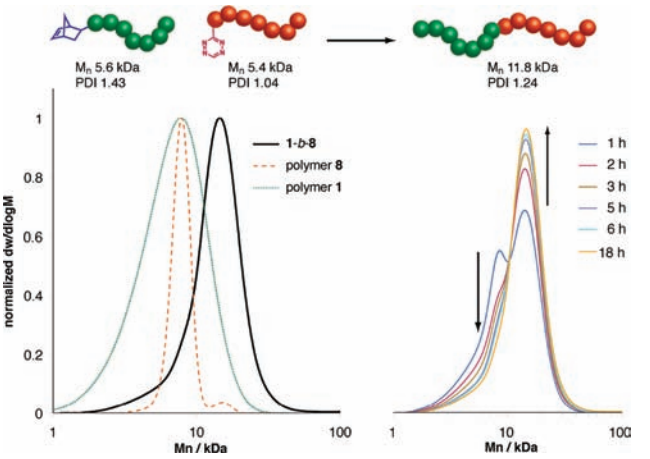

Figure 3. SEC traces of diblock PS-*b*-PEG and the constituent homopolymers (left), and the evolution of the PS-*b*-PEG conjugation SEC traces with time (right), showing disappearance of the homopolymer in parallel with appearance of the diblock peak.

to 95% conversion in 6 h in CH_2Cl_2 and within 4 h in CH_2Cl_2/DMSO, and more importantly, with high efficiency at equimolar ratios of functionality (Figure S15 in SI).

Evolution of the SEC traces with time provides an excellent method with which to follow this reaction, and SEC traces for the starting polymers and the clicked PEG-*b*-PS reveal that the resulting M_n values of the block copolymers are close to the addition of the initial polymers' M_n values, an important consideration when assessing polymer−polymer click (Figure 3, Table S2 [SI]). While merely evaluating the shape of the SEC distributions is often utilized for assessing the success or failure of a click conjugation between two polymers, a unimodal trace with a 'nice shape' is not enough to quantify the degree of conjugation,[32] although indeed such a unimodal distribution is displayed by the conjugated polymers.

With this in mind, a diffusion-ordered NMR spectroscopy (DOSY) experiment (Figure S17 [SI]) was utilized to analyze the final coupled polymer. The DOSY results, although non-quantitative, do indeed show coupling between the PS and PEG blocks, and the sample fits well to a single-population model with no evidence of uncoupled homopolymer detectable. Additionally, LCxSEC analysis was carried out (Figure S18 [SI]) in order to increase the detection threshold for any homopolymer present. In this case, a small amount of uncoupled PS was detected, but in amounts that are attributable to the presence of unfunctionalized and/or dead homopolymers, species which are to be expected in most RAFT polymerizations. The final block copolymer was also purified by repeated precipitation into methanol followed by extensive dialysis against deionized water, and the ^{1}H NMR spectra were analyzed to show that the expected block ratios were present (Figure S19 [SI]).

Given that the coupling of **1** and **8** proceeded with high efficiency according to several different analytical methods, we also attempted polymer−polymer coupling in water, using PNIPAM-Nb, **3** and **8**. SEC analysis revealed a clear shift in molecular weight (Figure S20 [SI]), although a slight low-molecular weight shoulder can be seen, which we attribute to the fact that **3** contained a higher proportion of dead or unfunctionalized chains than **1** (10% vs 5%). Couplings of a variety of PVL-Nb (5, 11, and 31 kDa) and PCL-Nb, **4**, with **8** were also carried out in CH_2Cl_2. Effective coupling was observed by SEC analysis for the ~5 and

10 kDa polymers coupled to the 5.4 kDa PEG-Tz, **8**; however for PVL-Nb, **7**, it was difficult to ascertain if coupling had been successful solely by SEC analysis on account of its higher molar mass (31 kDa) leading to the change in MW being small relative to that of the starting polymer. Using the SEC refractive index detector, it was observed that the PCL and PVL couplings appeared to be less efficient than the PS and PNIPAM couplings with **8**; we hypothesize that this may be because CH_2Cl_2 is not an optimum reaction solvent for these polymers. Nonetheless, monomodal traces by SEC-UV detection at ~320 nm (with no absorbance from uncoupled Tz at 546 nm) were observed at higher molecular weight than both of the starting homopolymers for all couplings attempted, showing that all Tz-functionalized homopolymer had reacted to form the relevant diblock copolymer (SEC traces shown in Figure S20 [SI]). PVL-Nb **5** and PVL-Tz **9** were also coupled to demonstrate that polymers grown from a tetrazine initiator are as effective in the reaction as polymers formed by postpolymerization modification.

In summary, we have demonstrated the utility and scope of the 'spring loaded' and additive-free tetrazine−norbornene click reaction as applied to a range of polymer−polymer conjugation and polymer end-functionalization in both water and organic solvents. We propose that this reaction offers some advantages over existing click methodologies for functionalization and coupling of polymers, particularly with regard to sensitive substrates or applications where external stimuli, catalysts, or reagents are not desirable. Currently, the limitations of this conjugation strategy lie primarily in the relative difficulty in the synthesis of the tetrazine starting materials, especially given their sensitivity under polymerization conditions. We are currently exploring the use of this reaction in a range of polymer and materials science applications.

■ ASSOCIATED CONTENT

Supporting Information. Synthesis and NMR, LC−MS, UV/vis, MALDI, DOSY, and LCxSEC data. This material is available free of charge via the Internet at http://pubs.acs.org.

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