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We have investigated the reversible hetero-Diels–Alder reaction of 1,2-oxazines derived from a peralkylcyclopentadiene and a series of nitrosocarbonyl dienophiles. The nature of the dieneophile was found to impart broad tunability to the dynamic character of the oxazine adducts. The reversibility was also observed in polymeric systems. The fidelity of the reaction and tunable sensitivity toward elevated temperature and water signify potential applications in the development of dynamic covalent materials or delivery systems for small molecule payloads.

Introduction

For decades, the hetero-Diels–Alder (HDA) reaction of nitrosocarbonyl species has been utilized as a versatile synthetic transformation for production of 1,2-oxazines (Fig. 1). Attractive aspects include the potential for regio- and stereo-controlled introduction of nitrogen and oxygen into carbon frameworks, and ample opportunities for downstream manipulation of the N-substituted oxazine. In a subset of examples of 1,2-oxazine applications, the ready reversibility of the HDA reaction was targeted as a means to control in situ generation of highly reactive nitrosocarbonyl moieties. For example, King, Miyata, and Toscano have highlighted the potential to use this cycloreversion for generation of HNO, a purported therapeutic, presumably following hydrolysis of the nitrosocarbonyl intermediate.

More recently, the dynamic HDA reaction of nitrosocarbonyls has been applied in the design of advanced polymeric architectures and functional materials. A key example from Read de Alaniz and coworkers demonstrated application of nitrosocarbonyl HDA reactions to prepare block copolymers via highly efficient polymer chain end coupling. In their report, reactive nitrosocarbonyl dienophiles were generated in situ at polymer chain ends either by oxidation of hydroxamic acids or cycloreversion of 9,10-dimethylanthracene-based adducts. In the presence of a complementary cyclopentadiene-terminated polymer, efficient chain end coupling was accomplished.

Inspired by this movement toward applications in the polymer field, we considered integration of polymer chain end coupling and nitrosocarbonyl hydrolysis to enable controlled deconstruction of block copolymers. For this purpose, the 1,2-oxazine moiety was employed as a thermally labile trigger. Upon thermolysis, subsequent hydrolysis of the nitrosocarbonyl intermediate initiated a controlled depolymerization sequence leading to deconstruction of a self-immolative polymer block. Clearly, the “click-like” nature of the HDA reaction, tunable cycloreversion of oxazines, diverse reactivity of nitrosocarbonyl species, and overall structural modularity provide a powerful combination for advances in polymer and materials designs.

We became interested in the potential applications of polymers and network materials bearing high densities of oxazine moieties. One may envision, for example, taking advantage of robust dynamic covalency to access covalent adaptable networks as has been demonstrated for Diels–Alder and HDA adducts. Such systems display broad tunability with regard to stimuli-responsiveness and overall materials properties, stemming largely from the modularity of the diene and dienophile building blocks. On the other hand, controlled...
breakdown of oxazine-rich materials could enable controlled release of HNO. HNO generation has received a lot of attention in recent years due to its purported use as a treatment of cardiovascular diseases and as an anticancer agent.\textsuperscript{22–26} Notably, the breadth of potential applications place disparate demands on the reactivity of the oxazine and constituent nitrosocarbonyl species (high fidelity cycloaddition/cycloreversion versus hydrolytic instability) while maintaining a need for retro-HDA reactions to take place at moderate temperatures. To further explore the chemical space and potential applications of oxazine-based systems, we have investigated the reversibility and robustness of a series of oxazines relevant to polymer-oriented applications. Herein, we describe our investigations of the reactivity of oxazines derived from a series of nitrosocarbonyl dienophiles and a peralkylcyclopentadiene. Particular focus is placed upon comparative analysis of the reactivity of oxazines derived from a series of analogous reactions with coupling partners \textsuperscript{4–6} were met with limited success. Turning instead to tetrabutylammonium periodate (TBAP) as a stoichiometric oxidant remedied the situation, providing the corresponding oxazines \textsuperscript{8–10} in good to excellent yields. Notably, each oxazine was isolated as a mixture of what appeared to be a roughly 3 : 1 ratio of two diastereomers (each racemic).

Separation of the isomers by standard chromatographic techniques was unsuccessful. However, we found that slow vapour diffusion of diethyl ether into solutions of \textsuperscript{10} in CH\textsubscript{3}Cl\textsubscript{2} produced single crystals suitable for X-ray analysis. We were able to separate crystals that were ultimately found to be individual diastereomers \textsuperscript{10a} and \textsuperscript{10b} (Fig. 3), which can be viewed as anti and syn isomers, respectively. With small quantities of each diastereomer of \textsuperscript{10} at hand, we were able to obtain a discrete \textsuperscript{1}H NMR spectrum for each. From these, we identified \textsuperscript{10a} as the major diastereomer produced in the initial mixture of products. The CH\textsubscript{3} groups indicated in Fig. 3 proved to be useful diagnostic handles for \textsuperscript{1}H NMR analyses and tracking of anti and syn isomers for oxazines \textsuperscript{8, 9, and 11} by analogy to \textsuperscript{10}.

We next turned toward probing the dynamic nature of each oxazine. Specifically, each set of isomers was heated in DMSO-\textsubscript{d\textsubscript{6}} (15 mM) and monitored by \textsuperscript{1}H NMR spectroscopy. In each case, the initial mixture contained a roughly 3 : 1 ratio of diastereomers, which equilibrated to a roughly 1 : 1 ratio over time. At ambient temperature, no observable isomerization took place for any of the oxazines over the course of several hours. When heated at 37 °C (Fig. 4, top), we observed gradual equilibration of the diastereomers with strong dependence upon the nature of the nitrosocarbonyl component. For example, oxazine \textsuperscript{8} did not appear to reach equilibrium even

\begin{figure}
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\includegraphics[width=\textwidth]{fig2.png}
\caption{Synthesis of norbornene-tethered oxazine isomers. In each case, reactions proceeded to 100% conversion as judged by TLC.}
\end{figure}
within 12 d at 37 °C, indicating much slower isomerization than oxazines bearing more electron donating substituents (cf. 9–11). Oxazine 9 appeared to isomerize more rapidly than 8, but also continued to show gradual change in the ratio of isomers up to 12 d. The hydroxyurea-derived systems (10 and 11) were found to approach equilibrium at the highest relative rates. The facile isomerization, particularly of 10 and 11, at 37 °C suggests to us that these platforms may be suitable for incorporation into dynamic covalent networks or related materials that become active under biological conditions.

The general trend in isomerization rate (8 < 9 < 10 < 11) was preserved at the higher temperatures as well (Fig. 4). At 60 °C (Fig. 4, middle), the least reactive oxazine (8) displayed discernible isomerization over the course of several days, whereas changes in the diastereomeric ratios for 10 and 11 appeared to have ceased after a few hours. Further increase in the temperature to 80 °C (Fig. 4, bottom) resulted in full equilibration within minutes for 10 and 11 (50 and 25 min, respectively), whereas the composition of 8 continued to show gradual change over the course of several hours (see ESI† for extended plots).

As stated previously, the oxazine retro-HDA reaction releases nitrosocarbonyl species, which have the potential to undergo hydrolysis, dimerization, or ene reactions.32 These deleterious reactions were not observed to any appreciable extent in the experiments represented in Fig. 4 despite prolonged reaction times and no special precautions being taken to dry the DMSO solvent. To explore the oxazine reactivity in aqueous environment, we examined each of the oxazines at 60 °C in a 30% D2O/DMSO-d6 mixture, which was the maximum D2O content at which each of the oxazines remained soluble at room temperature. Each oxazine mixture was monitored by 1H NMR spectroscopy for 156 h. Oxazines 8 and 9 showed no loss of oxazine content and isomerization rates similar to those observed in DMSO-d6. In contrast, examination of oxazine 10 revealed a small amount of cyclopentadiene 3 (4.5%) developing over the course of the experiment (Fig. 5, top). Presumably, the formation of 3 can be ascribed to
hydrolysis of the intermediate nitrosocarbonyl species. When oxazine 11 was examined under the aqueous reaction conditions, we found nearly complete loss of both oxazine isomers and concomitant formation of 3 (Fig. 5, bottom). Collectively, these results confirm that the oxazine moiety can be structurally tuned toward either robust dynamic behaviour or controlled degradation.

Encouraged by the results from the small molecule oxazine studies, we next explored whether the dynamic nature would remain consistent within related polymeric systems. Toward this end, we synthesized poly(11) via ring-opening metathesis polymerization (ROMP) using a third-generation Grubbs catalyst (Fig. 6). Specifically, monomer 11 was reacted with the Ru-based initiator (35:1 initial monomer to initiator ratio) in CH₂Cl₂ at 0 °C for 3 h, at which point full consumption of monomer was confirmed by ¹H NMR analysis. Following termination of the polymerization with ethyl vinyl ether, filtration through alumina/Celite, and isolation by precipitation of the polymer into methanol, we obtained poly(11) in 57% isolated yield. Analysis of poly(11) by ¹H NMR spectroscopy and gel-permeation chromatography (GPC) indicated intact oxazines (3:1 ratio of isomers), a weight-average molecular weight (Mₙ) of 30.0 kDa, and a molecular weight dispersity (Đ) of 1.05.

With poly(11) at hand, we then monitored the isomerization of the oxazine units using a solution of the polymer in DMSO-d₆ at 60 °C and variable-temperature ¹H NMR spectroscopy (VT-NMR). The alkene proton resonances within the polymer backbone were compared with the diagnostic methyl signals of the oxazine isomers over the course of the experiment. Under these conditions, poly(11) showed no signs of degradation of the oxazine units and complete isomerization within 5 h, consistent with the equilibration time of monomer 11 (Fig. 7).

To investigate the potential for dynamic nitrosocarbonyl exchange from the polymeric system, poly(11) was heated at 60 °C in the presence of either oxazine 9 or 10, each in a 2:1 ratio relative to oxazine repeat units in poly(11) (Fig. 8). After heating each mixture, the solution was added dropwise into an excess of cold methanol, causing selective precipitation of the polymeric species. In each case, ¹H NMR analysis of the final polymers revealed signals consistent with successful conversion of oxazine units, giving rise to poly(9-co-11) and poly(10-co-11) from the corresponding small molecule oxazines. Each copolymer was found to have a ca. 2:1 ratio of oxazine units consistent with the initial feed ratios. These experiments confirm the ability to successfully crossover oxazine functionality with a polymeric system, and may provide opportunities for post-polymerization modifications and reversible crosslinking strategies.

Conclusions

We have investigated a series of N-carbonyl-substituted oxazines that display readily reversible HDA reactivity. Coincidental production of a diastereomeric mixture of each oxazine
provided a convenient method for comparative analysis of their dynamic nature as a function of structure and temperature. The systems appeared to be relatively robust, with dynamic equilibration and preservation of oxazine content persisting for several hours at elevated temperatures. Moreover, we found encouraging results toward incorporation of these oxazines into dynamic covalent networks and release platforms based upon polymer-oriented and hydrolysis studies. Collectively, this series of compounds displays a broad range of (retro)HDA rates and general robustness that may help to guide the design of adaptable network materials and functional polymers.

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Notes and references

31 1,4-Dicyanobenzene was used as an internal NMR standard in the trials.