Copper-free click chemistry for the *in situ* crosslinking of photodegradable star polymers†

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Received (in Cambridge, UK) 21st February 2008, Accepted 25th March 2008
First published as an Advance Article on the web 24th April 2008
DOI: 10.1039/b803043j

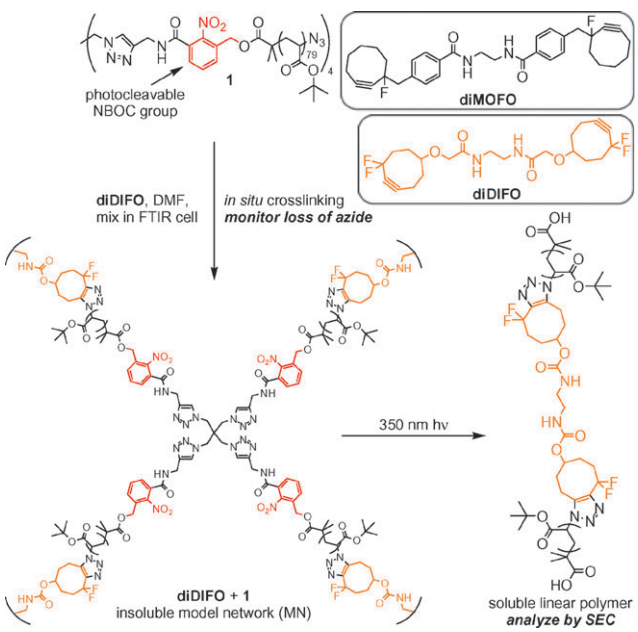
Bifunctional, fluorinated cyclooctynes were used for the *in situ* "click" crosslinking of azide-terminated photodegradable star polymers, yielding photodegradable polymeric model networks with well-defined structures and tunable gelation times.

Covalently crosslinked polymeric materials which swell, but do not dissolve, in a given solvent (polymer gels) have found utility in a number of applications including tissue engineering,[1a] drug delivery,[1b] chemical sensing,[1c] microfluidics,[1d] cosmetics,[1e] and microelectronics.[1f] End-linked polymer gels (model networks or MNs) are especially promising for drug delivery applications due to their easily controlled, homogeneous pore sizes.[2] As a result, much effort has been focused on developing general synthetic strategies capable of yielding functional MNs.

MN synthesis can be roughly divided into two stages: (1) synthesis of a macromonomer (MAC) precursor and (2) crosslinking. Viewing the synthetic process in this manner enables one to design a MN bearing complex functionality by first utilizing the well-developed tools of standard organic and polymer synthesis to prepare functional MACs.[3] Then, MN synthesis is reduced to finding a crosslinking reaction that proceeds in high yield and is chemoselective between the desired crosslinking functionalities, but orthogonal to all other functionalities present, *i.e.*, reactions which meet the standards of Sharpless' click chemistry.[4]

As an example of this two-stage strategy, we recently reported[2c] the design and synthesis of photodegradable MNs based on a tetra-nitrobenzyloxycarbonyl (NBOC), tetra-azido terminated, poly(*tert*-butyl acrylate (*t*BA)) star polymer (**1**, Scheme 1) which was prepared by tandem copper-catalyzed azide–alkyne cycloaddition[5] (CuAAC) and atom transfer radical polymerization (ATRP)[6] followed by end group transformation. The *t*BA monomer was chosen because it can be easily hydrolyzed to acrylic acid thus yielding hydrogels; however, using this approach, chemically diverse star MACs can be prepared from presumably any combination of the numerous monomers polymerizable by ATRP (acrylates, methacrylates, acrylamides, styrenics, *etc*...). For the crosslinking step, **1** was allowed to react with a bifunctional alkyne *via* CuAAC to yield MNs. As expected for the "cream of the crop"[4] of click reactions, CuAAC crosslinking was achieved chemoselectively in high yield.

Having shown the effectiveness of ATRP and CuAAC for preparing MACs, we chose to focus on optimizing the crosslinking reaction because, despite the success of CuAAC for the crosslinking of **1**, it has a few major drawbacks which hinder its general applicability to MN and polymer gel synthesis. First, to obtain the most homogeneous MNs in organic solvents, the crosslinking had to be performed under an inert atmosphere, a requirement not suitable for many industrial applications. Furthermore, the crosslinking required the use of copper as a catalyst, as well as a ligand/base additive, so repeated swelling of the materials in fresh solvent was necessary to yield "pure" materials. Also, copper is known to be cytotoxic to most bacterial and mammalian cells,[7] and because



Scheme 1 Structures of star polymer **1**, **diMOFO**, **diDIFO**, MN from **diDIFO** and **1**, and the corresponding linear polymer photodegradation product. To clearly depict their location in the MN structure, photodegradable NBOC groups are shown in red and **diDIFO** is shown in orange.

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† Electronic supplementary information (ESI) available: Experimental details. See DOI: 10.1039/b803043j

it is very difficult to ensure, even after extensive extraction, that all of the copper has been removed from the bulk gel, it is highly desirable to avoid CuAAC crosslinking altogether for biological applications. The toxicity of copper could also complicate the use of CuAAC for biological *in situ* crosslinking (*i.e.*, crosslinking *in vivo* or in the presence of living cells). Finally, CuAAC crosslinking led to rapid, uncontrolled gelation, and for certain applications it may be desirable to control the gelation time in order to synchronize with another process of interest.

In search of an alternative to CuAAC for crosslinking, it became necessary to decide whether a modification to **1** was necessary or if an alternative reaction of azides could be employed. As mentioned above, diversely functional polymers possessing azide end groups can be easily prepared by ATRP and end group modification.[2c,8] Furthermore, azides are known to be bioorthogonal.[9] For these reasons, we explored alternative reactions of azides for crosslinking rather than change to another functionality altogether. Fortunately, all of the drawbacks of CuAAC crosslinking described above can indeed be overcome by using a copper-free variant that utilizes fluorinated cyclooctyne reagents to effect Huisgen [3+2] dipolar cycloaddition with azides. This reaction, the strain-promoted azide–alkyne cycloaddition[9] (SPAAC), has been shown to proceed very efficiently with high chemoselectivity even in *in vivo* applications, making it perfectly suitable for *in situ* crosslinking. Furthermore, since no copper or ligand/base is required, there would be only two components to the network and, as a result, little extractable material. Finally, as has been shown previously, the rate of the SPAAC reaction can be controlled by making electronic modifications to the alkyne.[9b,c]

Carbodiimide-mediated condensation of ethylenediamine with two equivalents of monofluorinated cyclooctyne (MOFO) and difluorinated cyclooctyne (DIFO) yielded crosslinkers **diMOFO** and **diDIFO**, respectively (Scheme 1, see ESI†). DIFO is known to react with azides more rapidly in solution than MOFO[9c] and herein we report the same phenomenon during the crosslinking with **1**. Fourier transform infrared spectroscopy (FTIR) can be used to monitor the kinetics of the SPAAC reaction by monitoring the decay of the azide antisymmetric stretch absorption and assuming that its intensity is a linear function of the concentration of azide. Separate millimolar stock solutions in DMF of a known concentration of star polymer **1** and either **diMOFO** or **diDIFO** were mixed together such that the final concentration of azide : cyclooctyne was 1 : 1 in a total reaction volume of 10 μL. This solution was then quickly injected into the gelation cavity (a sealed, FTIR cell with CaF_2 plates and a 25 μm Teflon spacer), and spectra were recorded continuously until no change was observed in the azide absorbance at ~2100 cm^{-1}. Fig. 1 shows the decay of the azide absorbance over a 13 h time span, confirming the faster rate of azide loss for **diDIFO** *versus* **diMOFO** and thus confirming that the crosslinking kinetics can be controlled solely by altering the electronics of the crosslinker without modifying **1**. Unlike the SPAAC reactions of MOFO or DIFO with model azides in solution, which follow second order kinetics,[9b,c] these data display more complicated kinetic behavior indicative of the dynamically changing environment within the gelation cavity (*i.e.*, increasing viscosity) during crosslinking.

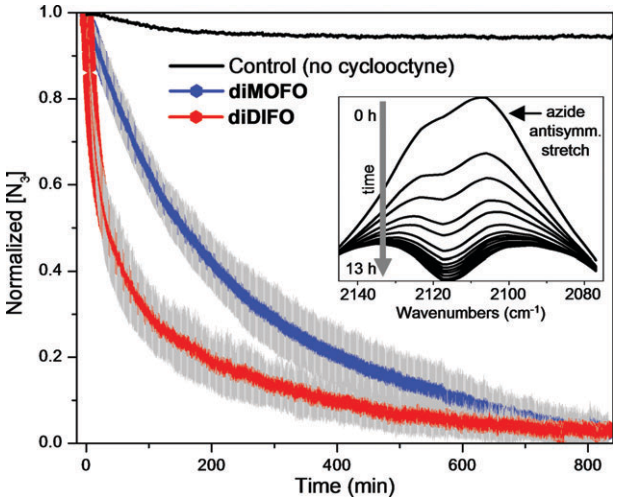

Fig. 1 Average decay curves for the loss of azide during gelation between **1** and **diMOFO** or **diDIFO** as monitored by FTIR. Experiments were performed in triplicate with error bars shown in grey. Inset shows the azide antisymmetric stretch region of the FTIR spectrum at different intervals during a typical gelation experiment with **diDIFO**.

We have shown previously that although no azide absorbance is observed after crosslinking, unreacted chains still inevitably exist due either to incomplete reaction or error in stoichiometry of azide : alkyne, and thus, FTIR is not sensitive enough to determine a yield for the crosslinking reaction.[2c,d] If crosslinking occurs chemoselectively, however, between the azide of **1** and the cyclooctyne of either crosslinker, well-defined MNs would result which, upon photocleavage of the NBOC groups, would yield soluble linear polymers having a number average molecular weight (M_n) equal to 0.5 that of **1** (Fig. 2, inset). Furthermore, unreacted chains would give soluble products having M_n equal to 0.25 that of **1**. To confirm chemoselective crosslinking, and to assess the yield of

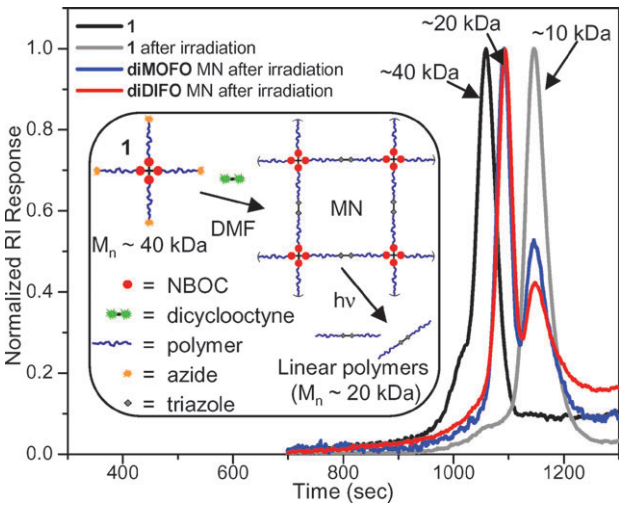

Fig. 2 SEC traces of **1** before and after photocleavage, and of the photodegradation products of **diMOFO** and **diDIFO** derived MNs. Inset shows schematic of crosslinking depicting the conversion of **1** to a MN and finally to linear polymers having $M_n \approx 0.5$ that of **1**.

crosslinking, the transparent gelation cavity was submerged in tetrahydrofuran (THF) and irradiated with 350 nm light for 2 d. The THF solution was then analyzed by size exclusion chromatography (SEC) and the resulting chromatograms are shown in Fig. 2. As expected, both MNs yielded a major peak with $M_n \approx 20$ kDa which corresponds to successfully crosslinked ends of 1. Both MNs also yielded a minor peak with $M_n \approx 10$ kDa corresponding to unreacted or "dangling" chain ends within the network. The MNs crosslinked with **diDIFO** showed fewer unreacted ends, a possible result of either its greater reactivity or, perhaps because it is smaller, its greater mobility in the highly hindered MN environment. Finally, the crosslinking yield for SPAAC is comparable to that found previously for CuAAC,[2c] confirming the high efficiency of the SPAAC reaction.

This study represents the first example of SPAAC in materials synthesis, specifically for the crosslinking of polymeric materials, and it opens a general route to complex, functional MNs capable of biocompatible, *in situ* crosslinking, controlled gelation time, and tailored degradation. Additionally, this work represents the first example of monitoring the kinetics of an *in situ* crosslinking process using the azide antisymmetric FTIR stretch, an approach which can be widely applied to studying the increasing repertoire of azide reactions.[10]

We thank the NSF (DMR-0214363, IGERT-0221589, DMR-0213574, CHE04-15516, graduate fellowship), the NIH (GM058867), the NDSEG (graduate fellowship), and the ACS Division of Organic Chemistry (graduate fellowship) for support of this work. We also thank J. Codelli and B. Dickinson for technical assistance.

Notes and references

1. (*a*) M. C. Cushing and K. S. Anseth, *Science*, 2007, **316**, 1133; (*b*) C.-C. Lin and A. T. Metters, *Adv. Drug Delivery Rev.*, 2006, **58**, 1379; (*c*) M. Guenther, D. Kuckling, C. Corten, G. Gerlach, J. Sorber, G. Suchaneck and K. F. Arndt, *Sens. Actuators, B*, 2007, **126**, 97; (*d*) N. Blow, *Nat. Methods*, 2007, **4**, 665; (*e*) J. Kopecek and J. Yang, *Polym. Int.*, 2007, **56**, 1078; (*f*) E. W. H. Jager, E. Smela and O. Inganas, *Science*, 2000, **290**, 1540.
2. (*a*) M. Achilleos, T. Krasia-Christoforou and C. S. Patrickios, *Macromolecules*, 2007, **40**, 5575; (*b*) M. Malkoch, R. Vestberg, N. Gupta, L. Mespouille, P. Dubois, A. F. Mason, J. L. Hedrick, Q. Liao, C. W. Frank, K. Kingsbury and C. J. Hawker, *Chem. Commun.*, 2006, 2774; (*c*) J. A. Johnson, M. G. Finn, J. T. Koberstein and N. J. Turro, *Macromolecules*, 2007, **40**, 3589; (*d*) J. A. Johnson, D. R. Lewis, D. D. Diaz, M. G. Finn, J. T. Koberstein and N. J. Turro, *J. Am. Chem. Soc.*, 2006, **128**, 6564.
3. C. J. Hawker and K. L. Wooley, *Science*, 2005, **309**, 1200.
4. H. C. Kolb, M. G. Finn and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2001, **40**, 2004.
5. (*a*) V. V. Rostovtsev, L. G. Green, V. V. Fokin and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2002, **41**, 2596; (*b*) C. W. Tornoe, C. Christensen and M. Meldal, *J. Org. Chem.*, 2002, **67**, 3057.
6. K. Matyjaszewski and J. Xia, *Chem. Rev.*, 2001, **101**, 2921.
7. A. J. Link, M. K. S. Vink, N. J. Agard, J. A. Prescher, C. R. Bertozzi and D. A. Tirrell, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 10180.
8. (*a*) B. S. Sumerlin, N. V. Tsarevsky, H. Gao, P. Golas, G. Louche, R. Y. Lee and K. Matyjaszewski, *ACS Symp. Ser.*, 2006, **944**, 140; (*b*) J.-F. Lutz, *Angew. Chem., Int. Ed.*, 2007, **46**, 1018.
9. (*a*) N. J. Agard, J. A. Prescher and C. R. Bertozzi, *J. Am. Chem. Soc.*, 2004, **126**, 15046; (*b*) N. J. Agard, J. M. Baskin, J. A. Prescher, A. Lo and C. R. Bertozzi, *ACS Chem. Biol.*, 2006, **1**, 644; (*c*) J. M. Baskin, J. A. Prescher, S. T. Laughlin, N. J. Agard, P. V. Chang, I. A. Miller, A. Lo, J. A. Codelli and C. R. Bertozzi, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**, 16793.
10. J. A. Prescher and C. R. Bertozzi, *Nat. Chem. Biol.*, 2005, **1**, 13.

