# Metal free thiol–maleimide 'Click' reaction as a mild functionalisation strategy for degradable polymers†

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**The stoichiometric reaction between thiols and maleimide-functional poly(ester)s is demonstrated to be a quantitative, tolerant, mild and efficient method for polymer modification.**

Aliphatic poly(ester)s are an important class of polymeric materials, especially in the fields of biomedicine and microelectronics, primarily a consequence of their excellent biocompatibility and biodegradability properties.[1] Amongst these polymers, poly(lactic acid), PLA, has received particular attention; however, approaches for the incorporation of functional handles are currently limited.[2] Recently, the 'Click' reaction concept has become important in efficient and robust polymer synthesis and modification.[3] These reactions, in which two complementary functional groups are 'clicked' together to provide a quantitative yielding reaction, should ideally be able to be applied under a wide range of mild conditions and be tolerant to many other functional groups. Undoubtedly the most commonly studied reaction of this family is the Cu(I) catalysed Huisgen 1,3-dipolar cycloaddition of azide and terminal alkyne functionalities. As a consequence of both its ease of use and high yielding reactions, this methodology has been widely applied for polymer modification and functionalisation including bioconjugation,[4] synthesis of block copolymers,[5] control of polymer architecture[6] and functionalisation of polymer structures,[7–9] potentially finding greater favour in the field as a consequence of the ability to apply the same Cu(I) catalysts for this reaction as for the atom transfer radical polymerisation (ATRP) process.[10]

This methodology has been developed to be a robust, efficient and tolerant method for modification of polymer structures, however, when applied to poly(ester) systems, degradation of the polymer backbone has been observed and has required either additional ω-end group capping[8] or the application of milder conditions resulting in extended reaction periods.[8,11] Furthermore, a major focus in the area of ring-opening polymerisation has been the development of metal-free catalyst systems, both organocatalysis and enzymatically catalysed processes.[12] Such approaches are considered to be advantageous as the requirement for the removal of toxic heavy metal impurities from the polymer products is not required, an important consideration for applications in biomedicine and microelectronics.

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Recent reports have begun to demonstrate the application of potentially suitable metal-free click reactions[13] including thiolene,[14] oxime[15] and reversible Diels–Alder conjugations.[16] However, our attention was drawn to the highly effective reaction between thiols and maleimides. This reaction is commonly applied in the field of bioconjugation[17] and thus maintains the acceptability of the process for biological applications. Notably, both Maynard[18] and Haddleton[9] and their co-workers have applied cysteine-reactive polymers synthesised by ATRP to conjugate peptides and proteins. Here, maleimide functional polymers have been applied to selectively target the thiol-containing cysteine residues in proteins and enzymes with excellent levels of selective conjugation being achieved;[17] this reaction is often catalysed by a tertiary amine such as $NEt_3$ in the reaction mixture. Herein we report our studies into the efficiency of this coupling chemistry, demonstrating it to be an efficient and tolerant methodology that is sufficiently mild to prevent any degradation of sensitive poly(ester) backbones.

Poly(lactic acid) bearing maleimide functional groups was synthesised by the ring-opening polymerisation of lactide catalysed by the previously reported thiourea–tertiary amine catalyst system.[19] In order to prevent any undesirable side reactions brought about by Michael addition of the activated chain-ends with the maleimide functionality, a furan protected alcohol functional maleimide, **1**, was employed as an initiator for the reaction (Fig. 1).[9] PLAs with targeted degrees of polymerisation of 20, 50 and 100 were obtained with complete end-group fidelity in 50, 120 and 240 min, respectively. Interestingly, while the furan protecting group is evident by $^1$H NMR spectroscopy, the mass peaks in the MALDI-ToF spectra of the protected maleimide functional PLAs correspond to those of the deprotected maleimide functionality, suggesting that this deprotection occurs readily upon ionisation by the laser. Upon removal of the residual catalytic species by filtration through a plug of silica gel, the

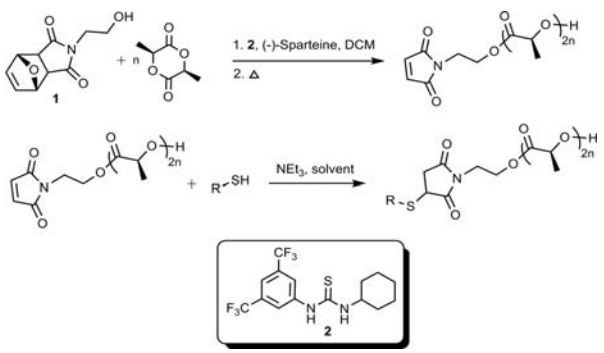


**Fig. 1** Synthesis of PLA and maleimide–thiol 'Click' reaction.

 

polymers were deprotected concurrently with drying in the solid state in a vacuum oven at 100 °C for 15 h. Comparison of the GPC traces of the polymers before and after deprotection reveal no significant changes, suggesting that they are not degraded by this process (DP20 protected: $M_n$ = 5470 g mol$^{-1}$, PDI = 1.07; DP20 deprotected (**3**): $M_n$ = 5240 g mol$^{-1}$, PDI = 1.09).

Initial investigations into the conjugation of thiol-containing molecules to these maleimide-functional PLAs (MI-PLA) focused on simple aliphatic and aromatic species (Fig. 1, Table 1). Upon treatment of the DP20 MI-PLA with 1.05 eq. thiophenol‡ and 1 eq. $NEt_3$, complete conversion was observed within 1 h. The reaction was stopped by precipitation of the polymer solution into hexanes. Examination of the $^1$H NMR spectra (Fig. 2b) reveals the disappearance of the characteristic alkene resonance at $\delta$ = 6.72 ppm and the appearance of aromatic signals at $\delta$ = 7.51 and 7.33 ppm, in addition to fully assignable resonances for the resultant succinimide functionality. Furthermore, the fully chain-end modified PLAs are observed in the MALDI-ToF spectrum (Fig. 2a). Further analysis of the MALDI-ToF spectrum reveals the absence of peaks spaced by 72 Da, and the polydispersity (measured by both MALDI-ToF and GPC) suggests that functionalisation of the polymer under these conditions does not lead to any undesirable transesterification side reactions. While mechanical losses of yield are observed as a consequence of the polymer precipitation, removal of solvent and excess reagents under vacuum resulted in isolation of the chain-end modified polymer in quantitative yield.

To more fully investigate the scope of this reaction, a selection of alkyl thiols were applied in the conjugation. In all cases these conjugations required extended periods of reaction. Indeed, reaction of DP20 MI-PLA with 1.05 eq. dodecanethiol (DDT) in the presence of 1 eq. $NEt_3$ had reached 53% conversion after 17 h. Further investigation of this reaction revealed that the conjugation

**Table 1** 'Click' conjugation of thiols to maleimide-functional PLAs[a]

| Entry | DP[b] | RSH | Time[c] | $M_n$[d] | PDI[d] |
|---|---|---|---|---|---|
| 1 | 20 | — | — | 5240 | 1.09 |
| 2 | 20 | PhSH | 40 min | 5410 | 1.08 |
| 3[e] | 20 | PhSH | <5 min | 5450 | 1.10 |
| 4[f,g] | 20 | $Me(CH_2)_{11}SH$ | 6 h | 5580 | 1.07 |
| 5[h] | 20 | $Me(CH_2)_{11}SH$ | 120 h | 4790 | 1.16 |
| 6[i] | 20 | $Me(CH_2)_{11}SH$ | 6 h | 3880 | 1.19 |
| 7[f,g,j] | 20 | $^i$PrSH | 6 h | 5290 | 1.09 |
| 8[f,g,j] | 20 | $^t$BuSH | 1 week | 5060 | 1.16 |
| 9 | 20 | $PhCH_2SH$ | 2 h | 5290 | 1.08 |
| 10 | 20 | Thioglycerol | 1.5 h | 4830 | 1.10 |
| 11 | 20 | $HOOC(CH_2)_2SH$ | 1.5 h | 5280 | 1.07 |
| 12 | 20 | Cysteamine | 1.5 h | 4860 | 1.07 |
| 13 | 20 | Cysteine ethyl ester | 1.5 h | 5470 | 1.06 |
| 14 | 50 | — | — | 13 600 | 1.05 |
| 15 | 50 | PhSH | 1 h | 13 600 | 1.05 |
| 16[k] | 50 | Glutathione | 48 h | 14 000 | 1.04 |
| 17 | 100 | — | — | 24 200 | 1.04 |
| 18 | 100 | PhSH | 1 h | 23 000 | 1.05 |
| 19[k] | 100 | Glutathione | 48 h | 22 500 | 1.07 |

[a] Reactions carried out in 7 mM THF solution of polymer, 1.05 eq. thiol and 1 eq. $NEt_3$. [b] Degree of polymerisation of maleimide functional PLA, measured by $^1$H NMR spectroscopy. [c] Time to reach >99.5% maleimide conversion as determined by $^1$H NMR spectroscopy. [d] Determined by GPC. [e] 2 eq. RSH. [f] 5 eq. RSH. [g] 2 eq. $NEt_3$. [h] 5 eq. $NEt_3$. [i] 10 eq. $NEt_3$. [j] Conjugations performed in DCM. [k] Conjugations performed in DMF at 40 °C.

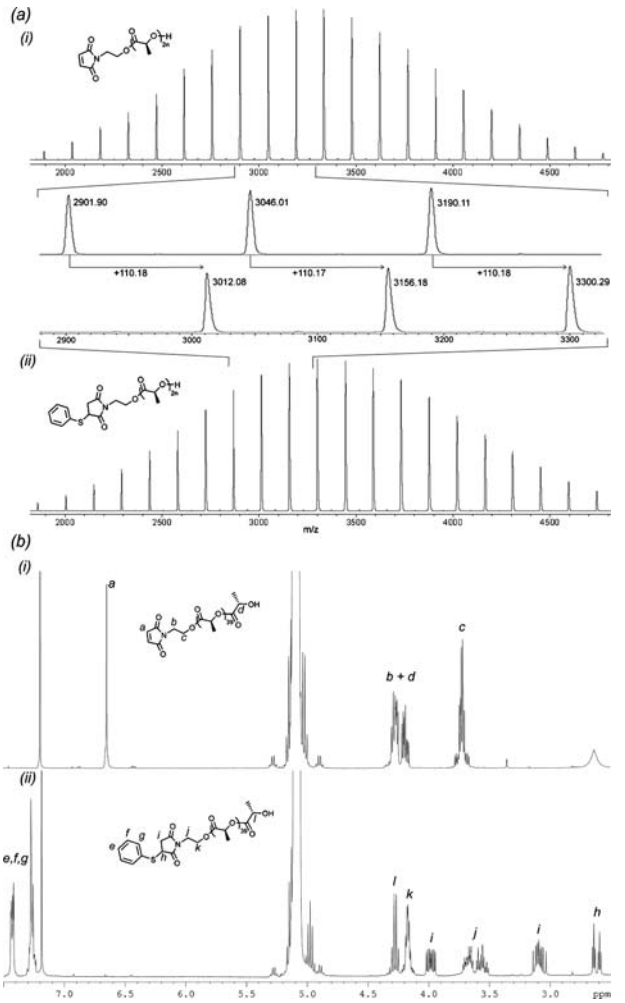


**Fig. 2** (a) MALDI-ToF spectra and (b) $^1$H NMR spectra ($CDCl_3$, 400 MHz) of DP20 PLAs (i) maleimide functional; (ii) thiophenol conjugated.

reaction can be significantly accelerated by increasing the equivalents of thiol (5 eq. DDT, 2 eq. $NEt_3$, 6 h) or by increasing the concentration of the triethylamine such that with 5 eq. and 10 eq. of $NEt_3$ to polymer/thiol, the reaction is completed within 120 and 6 h, respectively. Importantly, in all these cases no degradation of the polymer chain was observed by GPC and furthermore there was no evidence of transesterification in the MALDI-ToF spectra. Conjugation reactions have been carried out in a range of solvents; all solvents tested (DCM, THF, DMF, dioxane, acetone and DMSO (Table S1†)) were able to mediate the reactions, although it is worthy of note that laboratory grade DMSO led to consumption of the maleimide functionality without conjugation of the desired thiol, these side reactions can be overcome by using freshly distilled DMSO and are likely a result of thiol-based impurities in the solvent. In all cases, while the percentage conversions were modestly different, most likely a result of the accessibility of the polymer end-groups due to the coiling of polymer in solution, no degradation of the polymer chain was observed by MALDI-ToF, even upon addition of 1 or 2 vol% $H_2O$ to the medium.

Further extension of this methodology was aimed at showing the versatility of the conjugation method for a range of substituted and functional thiol-containing molecules. In DCM,

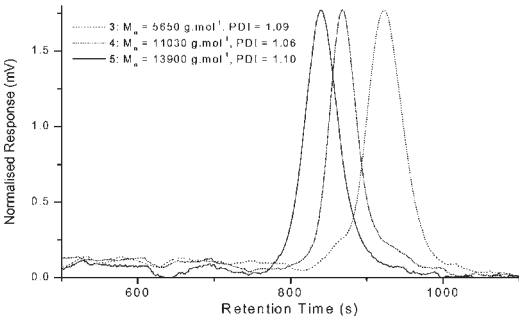


**Fig. 3** GPC traces of (a) DP20 MI-PLA, **3**; (b) telechelic (biphenyldithiol conjugate), **4**; (c) 3-arm star (1,3,5-(trithiomethyl)benzene conjugate), **5**.

$^{i}$PrSH and $^{t}$BuSH (5 eq.) were added to DP20 MI-PLA in the presence of 2 eq. $NEt_3$. Complete conversion to the thioester was observed after 6 h and 1 week, respectively. It is noteworthy however, that the requirement of a greatly extended conjugation time results in a slight high molecular weight shoulder in the GPC analysis, resulting in a broader PDI, indicative of transesterification side reactions. To determine the selectivity of the reaction, its tolerance towards functional groups was also examined and to this end 'Click' conjugations of thioglycerol, 3-mercaptopropionic acid, cysteamine, cysteine ethyl ester and glutathione, a simple tripeptide, were examined; suitable conditions were chosen to ensure compatibility of the thiol-containing molecule and PLA (see Table 1). In each case, the conjugation was observed to occur readily and the conjugations were all demonstrated to occur without degradation or transesterification of the PLA backbone by GPC and MALDI-ToF.

We have also applied this highly efficient methodology to demonstrate its versatility for the synthesis of telechelic and star-shaped PLAs. Application of biphenyl-4,4′-dithiol enabled clean conjugation of two MI-PLA chains to provide the resulting telechelic polymer, **4**. Given the difficulty of working at an exact 1 : 1 maleimide : thiol ratio, a slight excess of thiol was applied to completely consume the maleimide functionality.[5] Upon complete consumption of the maleimide functionality (monitored by $^1$H NMR spectroscopy) the solutions were treated with an iodoacetate resin to remove any excess thiol-containing residues.[20] After precipitation into hexanes and drying, GPC analysis of the polymers revealed that a highly efficient conjugation had been achieved without degrading the poly(ester) (DP20 MI-PLA, **3**: $M_n$ = 5240 g mol$^{-1}$, PDI = 1.09; **4**: $M_n$ = 11 030 g mol$^{-1}$, PDI = 1.06). Furthermore, extension of this methodology to realise star-shaped polymers was achieved by the application of 1,3,5-tri(thiomethyl)benzene in the conjugation reaction. This also resulted in isolation of a narrowly disperse star-shaped conjugated polymer, **5** (Fig. 3, Table S2†).

In summary, we have demonstrated that the application of maleimide–thiol coupling chemistry provides an efficient method for the modification of polymers. The process works efficiently in a range of solvents and conditions. Furthermore, its mild nature is demonstrated by application to poly(ester)s wherein no degradation of the polymer backbone is observed.

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## Notes and references

‡ 1.05 eq. thiol preferred in order to eliminate inaccuracies in measuring the small amounts required for the conjugation reactions and also uncertainty of polymer $M_n$ (determined by NMR spectroscopy). Stoichiometric reaction of thiophenol with small molecule maleimides revealed that the reaction is quantitative under these conditions.

1 A. C. Albertsson and I. K. Varma, *Biomacromolecules*, 2003, **4**, 1466–1486.
2 D. Bourissou, S. Moebs-Sanchez and B. Martin-Vaca, *C. R. Chim.*, 2007, **10**, 775–794.
3 J. F. Lutz, *Angew. Chem., Int. Ed.*, 2007, **46**, 1018–1025; C. J. Hawker, V. V. Fokin, M. G. Finn and K. B. Sharpless, *Aust. J. Chem.*, 2007, **60**, 381–383.
4 A. J. T. Dirks, S. S. van Berkel, N. S. Hatzakis, J. A. Opsteen, F. L. van Delft, J. Cornelissen, A. E. Rowan, J. C. M. van Hest, F. Rutjes and R. J. M. Nolte, *Chem. Commun.*, 2005, 4172–4174; N. S. Hatzakis, H. Engelkamp, K. Velonia, J. Hofkens, P. C. M. Christianen, A. Svendsen, S. A. Patkar, J. Vind, J. C. Maan, A. E. Rowan and R. J. M. Nolte, *Chem. Commun.*, 2006, 2012–2014; B. Le Droumaguet, G. Mantovani, D. M. Haddleton and K. Velonia, *J. Mater. Chem.*, 2007, **17**, 1916–1922.
5 J. A. Opsteen and J. C. M. van Hest, *Chem. Commun.*, 2005, 57–59; D. Quemener, T. P. Davis, C. Barner-Kowollik and M. H. Stenzel, *Chem. Commun.*, 2006, 5051–5053.
6 R. Hoogenboom, B. C. Moore and U. S. Schubert, *Chem. Commun.*, 2006, 4010–4012; H. Gao and K. Matyjaszewski, *J. Am. Chem. Soc.*, 2007, **129**, 11828–11834; H. F. Gao and K. Matyjaszewski, *J. Am. Chem. Soc.*, 2007, **129**, 6633–6639; B. A. Laurent and S. M. Grayson, *J. Am. Chem. Soc.*, 2006, **128**, 4238–4239.
7 J. A. Opsteen, R. P. Brinkhuis, R. L. M. Teeuwen, D. Lowik and J. C. M. van Hest, *Chem. Commun.*, 2007, 3136–3138.
8 R. Riva, P. Schmeits, F. Stoffelbach, C. Jerome, R. Jerome and P. Lecomte, *Chem. Commun.*, 2005, 5334–5336.
9 G. Mantovani, F. Lecolley, L. Tao, D. M. Haddleton, J. Clerx, J. Cornelissen and K. Velonia, *J. Am. Chem. Soc.*, 2005, **127**, 2966–2973.
10 G. Mantovani, V. Ladmiral, L. Tao and D. M. Haddleton, *Chem. Commun.*, 2005, 2089–2091.
11 X. Jiang, E. B. Vogel, M. R. Smith and G. L. Baker, *Macromolecules*, 2008, **41**, 1937–1944.
12 N. E. Kamber, W. Jeong, R. M. Waymouth, R. C. Pratt, B. G. G. Lohmeijer and J. L. Hedrick, *Chem. Rev.*, 2007, **107**, 5813–5840; I. K. Varma, A. C. Albertsson, R. Rajkhowa and R. K. Srivastava, *Prog. Polym. Sci.*, 2005, **30**, 949–981.
13 J. F. Lutz, *Angew. Chem., Int. Ed.*, 2008, **47**, 2182–2184.
14 K. L. Killops, L. M. Campos and C. J. Hawker, *J. Am. Chem. Soc.*, 2008, **130**, 5062–5064.
15 K. L. Heredia, Z. P. Tolstyka and H. D. Maynard, *Macromolecules*, 2007, **40**, 4772–4779.
16 A. Dag, H. Durmaz, G. Hizal and U. Tunca, *J. Polym. Sci., Part A: Polym. Chem.*, 2008, **46**, 302–313; H. Durmaz, B. Colakoclu, U. Tunca and G. Hizal, *J. Polym. Sci., Part A: Polym. Chem.*, 2006, **44**, 1667–1675; B. Gacal, H. Durmaz, M. A. Tasdelen, G. Hizal, U. Tunca, Y. Yagci and A. L. Demirel, *Macromolecules*, 2006, **39**, 5330–5336; S. Sinnwell, A. J. Inglis, T. P. Davis, M. H. Stenzel and C. Barner-Kowollik, *Chem. Commun.*, 2008, 2052–2054.
17 M. J. Roberts, M. D. Bentley and J. M. Harris, *Adv. Drug Delivery Rev.*, 2002, **54**, 459–476; F. M. Veronese, *Biomaterials*, 2001, **22**, 405–417.
18 Z. P. Tolstyka, J. T. Kopping and H. A. Maynard, *Macromolecules*, 2008, **41**, 599–606.
19 A. P. Dove, R. C. Pratt, B. G. G. Lohmeijer, R. M. Waymouth and J. L. Hedrick, *J. Am. Chem. Soc.*, 2005, **127**, 13798–13799; R. C. Pratt, B. G. G. Lohmeijer, D. A. Long, P. N. P. Lundberg, A. P. Dove, H. B. Li, C. G. Wade, R. M. Waymouth and J. L. Hedrick, *Macromolecules*, 2006, **39**, 7863–7871.
20 M. Li, P. De, S. R. Gondi and B. S. Sumerlin, *J. Polym. Sci., Part A: Polym. Chem.*, 2008, **46**, 5093–5100.

 