An approach to biodegradable star polymeric architectures using disulfide coupling†

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The straightforward synthesis of biodegradable star polymers *via* both *in situ* polymerization from a trifunctional RAFT agent and post-polymerization conjugation of pyridyldisulfide-ended linear polymers to a trithiol precursor is described.

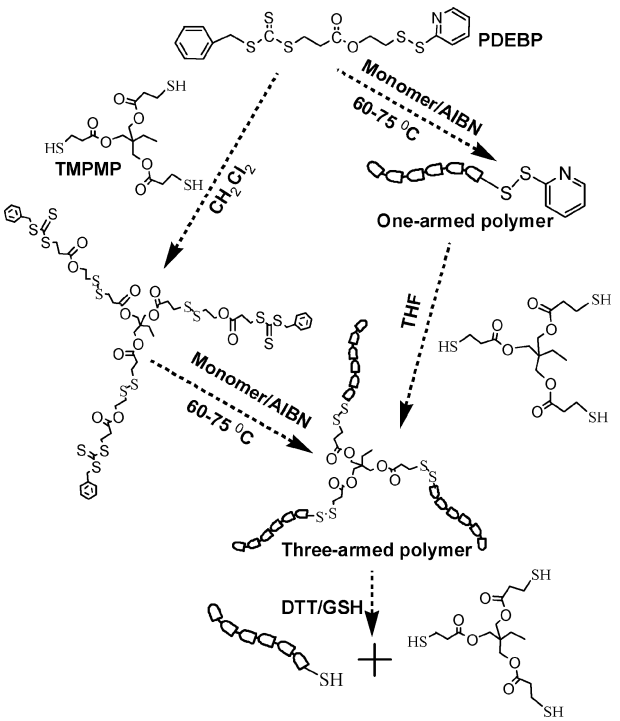
Multi-armed star polymeric architectures have attracted increasing interest due to their potential applications in a number of areas, *e.g.* encapsulation, sensing, catalysis, electronics, optics, biological engineering, coatings, additives, drug- and gene delivery.[1,2] Generally speaking, these complicated architectures could be achieved by 'arm-first'[3–6] or 'core-first' methodologies. Recently, the more straightforward "core-first" strategy has drawn an increasing interest for generating multi-armed polymeric architectures in a more controllable mode.[7–11] Star polymers consisting of miktoarms have also been tailored to achieve different properties.[6,12,13]

In addition to ionic and coordination ring-opening polymerization,[14,15] living radical polymerizations (LRPs) *e.g.* atom transfer radical polymerization (ATRP),[16,17] nitroxide-mediated radical polymerization (NMRP)[18] and reversible addition fragmentation chain transfer (RAFT) polymerization[19–21] have been exploited extensively to generate multi-armed structures with predetermined molecular weights and narrow molecular weight distributions. The combination of different polymerization methods were also used for generation of more complicated polymeric architectures.[3,13]

Most multi-armed polymeric structures have their arms joined to a core by stable covalent bonding. Therefore it is usually difficult to cleave the arms under mild conditions. The creation of complex polymeric structures with degradable linkages, (*e.g.* disulfide is cleavable in the presence of GSH[22–24] and acetal is acid labile[25]) is of great importance in biological applications. The disulfide bond is a reversible linkage that exists ubiquitously in proteins and enzymes. It is biodegradable *in vivo* in the presence of glutathione (GSH), the most abundant intracellular thiol (0.2–10 mM) in most mammalian and many prokaryotic cells.[22] Therefore a polymeric structure intra-linked by disulfide bonding could be easily cleaved *in vivo* and excreted subsequently over a much shorter period compared with its precursor.

Linear polymers and hydrogels with pyridyldisulfide (PDS) ended groups or disulfide intra-linkages have been extensively studied and utilized for the synthesis of functional polymers and biomolecule–polymer conjugates.[23,24,26–30] However, to the best of our knowledge, the well-defined multi-armed star architecture containing disulfide intra-linkages has not been reported yet. In this communication we report the direct synthesis of three-arm star polymeric architectures using both "core-first" and "arm-first" methodologies to generate a three-armed architecture containing biodegradable disulfide linkages, as outlined in Scheme 1. In the 'core-first' approach a trifunctional initiator was first synthesized by covalently attaching a trithiocarbonate RAFT agent, 3-[pyridyldisulfide]-ethyl, 3-benzyltrithiocarbonate propionate (PDEBP) to a trithiol precursor, trimethylolpropane tris[3-mercaptopropionate] (TMPMP) through disulfide coupling *via* their z-group, followed by the direct chain propagation from the RAFT core to afford three-armed star polymers. The same structure could also be achieved by 'arm-first' methodology, where a linear polymeric chain with PDS functionality was synthesized first using PDEBP, followed by the covalent coupling to TMPMP *via* disulfide linkage.

The three-armed RAFT agent was first characterized by ^{1}H NMR. As shown in Fig. 1 the disappearance of the peaks at



Scheme 1 'Core-first' and 'arm-first' methodologies to generate identical three-armed polymers and the subsequent cleavage into single-armed linear chains.

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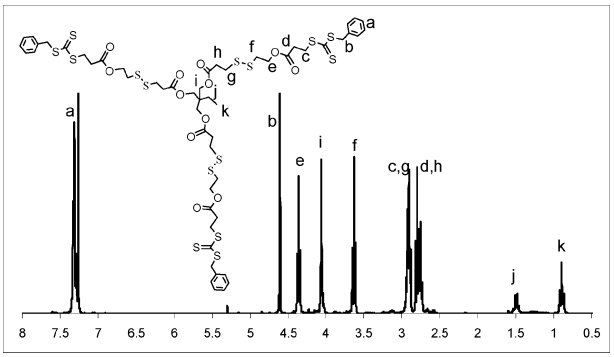

Fig. 1 ^{1}H NMR of three-armed RAFT agent.

8.49, 7.67 and 7.11 ppm from the PDS groups on PDEBP precursor (Fig. S1, ESI†) evidenced the successful coupling of the RAFT to trithiol precursor through the thiol–disulfide exchange reaction. The ratio of integration of protons from the phenyl groups at 7.33 ppm to those from TMPMP confirmed that each TMPMP molecule attached three RAFT molecules. The successful synthesis of the three-armed RAFT core was also supported by the presence of a parent sodium ion at m/z 1411.07 (calc. 1411.04) in its electrospray ionization (ESI) mass spectrum. (Fig. S2, ESI†)

The direct polymerizations of NIPAAm and styrene using the three-armed RAFT core proved to be well controlled by RAFT mechanism. As shown in Fig. S3(a,b)† and Fig. S4(a,b)† the monomer conversion increased with increasing polymerization time and the radical concentration was constant during the whole polymerization as indicated by pseudo-first order plot. The polydispersity indices (PDI) for purified PNIPAAm and PSt were 1.28 and 1.24, respectively. The low PDI values and the well-defined chromatograms from gel permeation chromatography (GPC) analysis strongly suggested that the chain propagation on the three arms was synchronous. The NMR signals from the RAFT residue could be observed clearly in the ^{1}H NMR spectra of purified PNIPAAm and PSt in Fig. S3(c)† and Fig. S4(c)†, evidencing the integrity of RAFT moieties after polymerization.

The cleavability of three-armed PNIPAAm was first checked by DL-dithiothereitol (DTT). 10 mM PNIPAAM was cleaved into one-armed chains in 2 hours in 0.1 M DTT aq. solution (pH 6.5). (Fig. 2a). After cleavage, the PDI of the single-armed chain decreased to 1.20, furnishing strong evidence for successful living polymerization. The M_w of the single-armed chain after cleavage was calculated to be 3800 from GPC, which is approximately one third of its precursor. The bio-degradability of three-armed PNIPAAm was tested by incubating 5 mg polymer in GSH solution (40 mM in 1 ml pH 5.0 phosphate buffer). After 48 hours, 30% of three-armed polymer was found to be cleaved (Fig. S5, ESI†). Apparently, the disulfide-cleavability of GSH was much weaker than that of DTT. The adoption of lower pH was based on the principle that lower pH will increase the reduction potential of GSH according to the Nernst equation.[31]

The 'arm-first' methodology was also employed to synthesize the identical three-armed structure. One-armed linear PSt with terminal PDS groups was sythesized first, followed by covalent coupling to TMPMP. GPC traces indicated an increase of M_w after conjugation. (Fig. 2b) The M_w of the

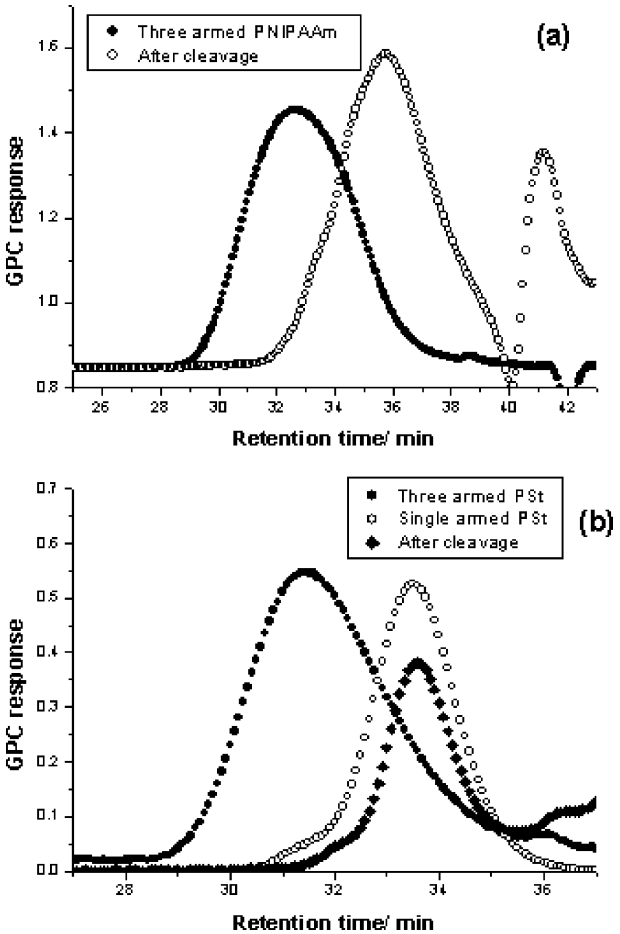

Fig. 2 (a) GPC traces of three-armed PNIPAAm (M_w 10 000 from ^{1}H NMR, PDI 1.28) (filled circles) and cleaved mixture in 0.1 M DTT (empty circles) and (b) GPC traces of linear PSt (empty circles), conjugation mixture with TMPMP (filled circles) and conjugation mixture after cleavage in 0.1 M DTT for 2 h (filled diamonds).

star was calculated to be 2.3 times of its one-armed precursors. This observation is consistent with the result obtained from theoretical simulation.[32] The star polymer was cleaved in the presence of DTT. After cleavage, the molecular weight distribution overlapped with that of the original one-armed precursor, consistent with complete cleavage. The covalent coupling of the linear polymeric chain to the trithiol core was monitored by UV-Vis spectroscopy. Upon reaction with TMPMP, the release of 2-pyridinethione was observed clearly at 370 nm, its characteristic absorption wavelength in dimethyl acetamide.[33] (Fig. S6, ESI†).

The coupling reaction of linear PNIPAAm with TMPMP was also characterized using ESI mass spectroscopy. The mass spectrum of linear PNIPAAm (M_n 820 from ^{1}H NMR, PDI 1.12) is shown in Fig. 3a. After coupling the M_w of the star polymer increased significantly. (Fig. 3b) It should be noted that any three of the linear chains could couple to one TMPMP to form a three-armed structure. However the three-armed structure formed by the same linear chains was still identified from the spectrum of the reaction mixture, as shown in Fig. 3.

In summary, we have successfully demonstrated the synthesis of bio-degradable three-armed polymers using both

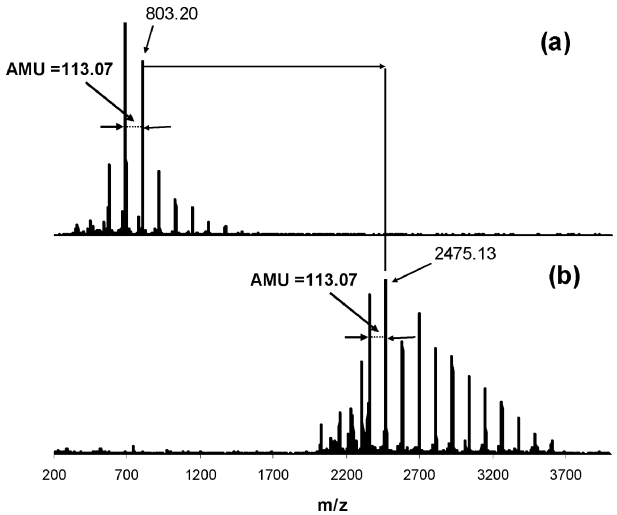

Fig. 3 ESI mass spectra of one-armed PNIPAAm terminated with PDS groups (a) and the three-armed star polymer (b).

"core-first" and "arm-first" methodologies. These biodegradable, multi-armed polymers are very promising for fabrication of conjugates using a variety of biomolecules such as proteins, peptides, oligoneucleotides, si-RNA, and genes for the applications in drug delivery and biotherapeutics. This simple methodology can easily be extended to higher-armed structures by the judicious selection of functional cores.

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Notes and references

1 N. Hadjichristidis, M. Pitsikalis, S. Pispas and H. Iatrou, *Chem. Rev.*, 2001, **101**, 3747–3792.
2 K. Inoue, *Prog. Polym. Sci.*, 2000, **25**, 453–571.
3 C. T. Adkins and E. Harth, *Macromolecules*, 2008, **41**, 3472–3480.
4 H. T. Lord, J. F. Quinn, S. D. Angus, M. R. Whittaker, M. H. Stenzel and T. P. Davis, *J. Mater. Chem.*, 2003, **13**, 2819–2824.
5 L. A. Connal, R. Vestberg, C. J. Hawker and G. G. Qiao, *Macromolecules*, 2007, **40**, 7855–7863.
6 J. Zhu, X. L. Zhu, E. T. Kang and K. G. Neoh, *Polymer*, 2007, **48**, 6992–6999.
7 M. H. Stenzel-Rosenbaum, T. P. Davis, V. K. Chen and A. G. Fane, *Macromolecules*, 2001, **34**, 5433–5438.
8 M. Stenzel-Rosenbaum, T. P. Davis, V. Chen and A. G. Fane, *J. Polym. Sci., Part A: Polym. Chem.*, 2001, **39**, 2777–2783.
9 M. H. Stenzel and T. P. Davis, *J. Polym. Sci., Part A: Polym. Chem.*, 2002, **40**, 4498–4512.
10 J. Bernard, X. J. Hao, T. P. Davis, C. Barner-Kowollik and M. H. Stenzel, *Biomacromolecules*, 2006, **7**, 232–238.
11 C. Y. Hong, Y. Z. You, J. Liu and C. Y. Pan, *J. Polym. Sci., Part A: Polym. Chem.*, 2005, **43**, 6379–6393.
12 A. Hirao, T. Higashihara and K. Inoue, *Macromolecules*, 2008, **41**, 3579–3587.
13 C. Liu, Y. Zhang and J. L. Huang, *Macromolecules*, 2008, **41**, 325–331.
14 L. R. Hutchings, *Soft Matter*, 2008, **4**, 2150–2159.
15 M. Spinu, C. Jackson, M. Y. Keating and K. H. Gardner, *J. Macromol. Sci., Pure Appl. Chem.*, 1996, **33**, 1497–1530.
16 K. Matyjaszewski and J. H. Xia, *Chem. Rev.*, 2001, **101**, 2921–2990.
17 L. Tao, G. Mantovani, F. Lecolley and D. M. Haddleton, *J. Am. Chem. Soc.*, 2004, **126**, 13220–13221.
18 C. J. Hawker, A. W. Bosman and E. Harth, *Chem. Rev.*, 2001, **101**, 3661–3688.
19 R. T. A. Mayadunne, J. Jeffery, G. Moad and E. Rizzardo, *Macromolecules*, 2003, **36**, 1505–1513.
20 L. Barner, T. P. Davis, M. H. Stenzel and C. Barner-Kowollik, *Macromol. Rapid Commun.*, 2007, **28**, 539–559.
21 V. Darcos, A. Dureault, D. Taton, Y. Gnanou, P. Marchand, A. M. Caminade, J. P. Majoral, M. Destarac and F. Leising, *Chem. Commun.*, 2004, 2110–2111.
22 M. E. Anderson, *Chem.-Biol. Interact.*, 1998, **112**, 1–14.
23 C. M. Li, J. Madsen, S. P. Armes and A. L. Lewis, *Angew. Chem., Int. Ed.*, 2006, **45**, 3510–3513.
24 R. Nicolay, L. Marx, P. Hemery and K. Matyjaszewski, *Macromolecules*, 2007, **40**, 9217–9223.
25 Y. Chan, T. Wong, F. Byrne, M. Kavallaris and V. Bulmus, *Biomacromolecules*, 2008, **9**, 1826–1836.
26 K. L. Heredia, T. H. Nguyen, C. W. Chang, V. Bulmus, T. P. Davis and H. D. Maynard, *Chem. Commun.*, 2008, 3245–3247.
27 J. Liu, V. Bulmus, C. Barner-Kowollik, M. H. Stenzel and T. P. Davis, *Macromol. Rapid Commun.*, 2007, **28**, 305–314.
28 J. Q. Liu, V. Bulmus, D. L. Herlambang, C. Barner-Kowollik, M. H. Stenzel and T. R. Davis, *Angew. Chem., Int. Ed.*, 2007, **46**, 3099–3103.
29 C. Boyer, V. Bulmus, J. Q. Liu, T. P. Davis, M. H. Stenzel and C. Barner-Kowollik, *J. Am. Chem. Soc.*, 2007, **129**, 7145–7154.
30 L. J. Wong, C. Boyer, Z. F. Jia, H. M. Zareie, T. P. Davis and V. Bulmus, *Biomacromolecules*, 2008, **9**, 1934–1944.
31 F. Q. Schafer and G. R. Buettner, *Free Radical Biol. Med.*, 2001, **30**, 1191–1212.
32 C. L. Fu, W. Ouyang, Z. Y. Sun and L. An, *J. Chem. Phys.*, 2007, **127**, 044903.
33 G. T. Hermanson, *Bioconjugate Techniques*, Academic Press, New York, 1996.

