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Facile synthesis of thiol-functionalized amphiphilic polylactide–methacrylic diblock copolymers†

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Biodegradable amphiphilic diblock copolymers based on an aliphatic ester block and various hydrophilic methacrylic monomers were synthesized using a novel hydroxyl-functionalized trithiocarbonate-based chain transfer agent. One protocol involved the one-pot simultaneous ring-opening polymerization (ROP) of the biodegradable monomer (3*S*)-*cis*-3,6-dimethyl-1,4-dioxane-2,5-dione (L-lactide, LA) and reversible addition–fragmentation chain transfer (RAFT) polymerization of 2-(dimethylamino)ethyl methacrylate (DMA) or oligo(ethylene glycol) methacrylate (OEGMA) monomer, with 4-dimethylaminopyridine being used as the ROP catalyst and 2,2′-azobis(isobutyronitrile) as the initiator for the RAFT polymerization. Alternatively, a two-step protocol involving the initial polymerization of LA followed by the polymerization of DMA, glycerol monomethacrylate or 2-(methacryloyloxy)ethyl phosphorylcholine using 4,4′-azobis(4-cyanovaleric acid) as a RAFT initiator was also explored. Using a solvent switch processing step, these amphiphilic diblock copolymers self-assemble in dilute aqueous solution. Their self-assembly provides various copolymer morphologies depending on the block compositions, as judged by transmission electron microscopy and dynamic light scattering. Two novel disulfide-functionalized PLA-branched block copolymers were also synthesized using simultaneous ROP of LA and RAFT copolymerization of OEGMA or DMA with a disulfide-based dimethacrylate. The disulfide bonds were reductively cleaved using tributyl phosphine to generate reactive thiol groups. Thiol–ene chemistry was utilized for further derivatization with thiol-based biologically important molecules and heavy metals for tissue engineering or bioimaging applications, respectively.

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Introduction

Block copolymers based on the same type of monomer (*e.g.* either vinyl or cyclic monomers) are traditionally prepared using a single "living" polymerization technique.[1] Recently, novel block copolymers with interesting properties have been prepared by combining two or more "living" polymerization chemistries to copolymerize dissimilar monomers.[2–33] Sequential polymerizations are most commonly used for such syntheses.[2–18] In principle, simultaneous polymerization can also lead to the synthesis of block copolymers. In practice, there are some examples in the literature for which incompatibility problems have been overcome to combine different polymerization techniques for the synthesis of well-defined block copolymers in a single step.[19–33]

The combination of ring-opening polymerization (ROP)[34] for the controlled synthesis of biodegradable aliphatic polyesters[35,36] and reversible addition-fragmentation chain transfer (RAFT) polymerization,[37–40] allows the synthesis of defined block copolymer architectures (for a wide range of vinyl monomers).[9,11,15,41–44] This approach bodes well for the synthesis of block copolymers for biomedical applications such as sutures, implants for bone fixation, drug delivery vehicles and tissue engineering scaffolds.[45]

A typical sequential polymerization strategy for the synthesis of polylactide (PLA)-based block copolymers is either to introduce a RAFT agent after the ROP of lactide (LA)[2,10] or to initiate ROP of LA after RAFT polymerization (*e.g.* from a hydroxyl functionality of a monomer previously polymerized by RAFT).[8] An alternative approach is the use of a bifunctional agent, *i.e.* a RAFT chain transfer agent (CTA) bearing a hydroxyl functional group either in the *R*- or in the *Z*-position[41] that can initiate both polymerizations. This CTA is either used to mediate the radical process followed by a second step in which the hydroxyl group initiates the ROP of LA,[7,13,16] or ROP is performed first, followed by RAFT polymerization.[6,12,14,17] Usually, an intermediate

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purification step is required. Clearly, simplifying the process to just one step where the two polymerizations proceed simultaneously is desirable. Exploring the simultaneous one-pot ROP–RAFT polymerization of LA with a vinyl monomer appears to be promising for the synthesis of new amphiphilic biodegradable PLA-based copolymers. Previous studies have focused on the simultaneous one-pot ROP of other monomers such as ε-caprolactone,[21–23,30,33] δ-valerolactone,[22,33] trimethylene carbonate,[22] β-butyrolactone[28] and RAFT polymerization, and also the simultaneous ROP of LA and RAFT polymerization of acrylic monomers.[24,29] Simultaneous ROP of LA and RAFT polymerization of methacrylic monomers for the preparation of biodegradable PLA-containing block copolymers using a metal-free approach[46] has been previously described, but mainly in the context of the synthesis of *hydrophobic* diblock copolymers.[22] As far as we are aware, such an approach has not been explored for the preparation of amphiphilic PLA-based block copolymers, particularly when the water-soluble block is based on methacrylic repeat units.

Amphiphilic diblock copolymers have attracted the interest of many researchers because they undergo spontaneous self-assembly in aqueous solution. This results in the formation of various morphologies such as spherical micelles, worm-like micelles, vesicles or intermediate structures.[47,48] Often the relatively high molecular weight (MW) and/or glass transition temperature can hinder the direct dissolution of amphiphilic diblock copolymers in water, since this makes the formation of micelles or vesicles extremely slow and inefficient.[49] These kinetic constrains can be overcome using either thin film rehydration or by prior dissolution using water-miscible co-solvents. This latter approach, commonly known as the 'solvent switch' method, is widely used for block copolymer self-assembly.[50] Here, the block copolymer is initially dissolved in a water-miscible common organic solvent for both copolymer blocks. Then water is added gradually to the copolymer solution resulting in the formation of various nanostructures after removal of the organic solvent.[47] One potential application for amphiphilic copolymers is in drug delivery. Here it is often considered desirable to conjugate peptides, vitamins or sugars so as to confer cell/tissue specificity and targeting.

Amongst the various conjugation approaches, thiol–disulfide chemistry has been widely used for biomedical applications[51–54] because of its orthogonality, reversibility and redox activity.[55–60] The reduction of a disulfide bond results in the formation of thiol functional groups. The presence of thiol functionalities during a radical-based vinyl polymerization such as RAFT is not desirable since they can act as efficient chain transfer agents.[61] We have previously reported post-polymerization formation of thiol-functional copolymers using a disulfide-based dimethacrylate (DSDMA) comonomer.[62–66] This disulfide acts as an atom-efficient thiol-protecting group for the synthesis of branched methacrylic copolymers. In relatively dilute solution, DSDMA undergoes predominantly intramolecular cyclization on its statistical copolymerization with a methacrylic monomer.[63] Thus cleavage of the lightly branched copolymer results in the formation of near-monodisperse copolymer chains[62,64,65] bearing thiol functionality.

The purpose of this work is to investigate the one-pot metal-free ROP–RAFT synthesis of biocompatible linear and branched amphiphilic diblock copolymers based on a biodegradable aliphatic polyester (PLA) and methacrylic monomers. The branched diblock copolymers are prepared using DSDMA as a comonomer for the methacrylic block. Its disulfide bond can be reductively cleaved to produce a thiol-functionalized amphiphilic block copolymer. In principle, such thiols can be useful for conjugation of various unsaturated molecules by thiol–ene chemistry, which has been widely used in polymer science.[60,67–69] This approach is especially advantageous for biologically relevant molecules such as oligopeptides since no cytotoxic metal catalysts are required. We also investigate a two-step protocol for preparation of PLA-based amphiphilic block copolymers. This approach can be applied to copolymers that cannot be synthesized *via* the one-step protocol, such as when the ROP conditions are incompatible with those of RAFT polymerization. For example, hydroxyl-functional monomers can act as ROP initiators, while biomimetic methacrylic monomers such as 2-(methacryloyloxy)-ethyl phosphorylcholine are only soluble in protic solvents, which are unsuitable for ROP. Finally, the self-assembly behavior of selected block copolymers is examined in aqueous solution.

Experimental section

Materials

2-(Methacryloyloxy)ethyl phosphorylcholine (MPC, >99.9%) was kindly donated by Biocompatibles UK, Ltd. (Farnham, UK). Oligo(ethylene glycol)methacrylate (OEGMA) and glycerol monomethacrylate (GMA, 97%) were kindly donated by Cognis UK Ltd. (Hythe, UK). THF (HPLC grade), DMF (chromatography GPC grade), chloroform ($CHCl_3$, HPLC grade), methanol (CH_3OH, HPLC grade), *n*-hexane (99.8%, Certified ACS), ethyl acetate (HPLC grade) and triethylamine (Et_3N, laboratory reagent grade, ≥99%) were purchased from Fisher Scientific (Loughborough, UK). 2-(Dimethylamino)ethyl methacrylate (DMA, 98%), (3*S*)-*cis*-3,6-dimethyl-1,4-dioxane-2,5-dione (L-lactide, LA, 98%), 4-cyano-4-(phenylcarbonothioylthio) pentanoic acid (CADB), 4,4′-azobis(4-cyanovaleric acid) (ACVA), hexane-1,6-diol (99%), dimethylaminopyridine (DMAP), 1,2-dichloroethane (anhydrous, 99.8%), anhydrous ethanol (≥99.5%), tributylphosphine (Bu_3P, mixture of isomers 97%), divinyl sulfone (DVS, 97%), tris(2-carboxyethyl)phosphine hydrochloride (TCEP, purum, ≥98.0%), L-glutathione (Glu, reduced form, ≥99%) and indium(III) chloride ($InCl_3$, 99.999% trace metals basis) were purchased from Sigma-Aldrich (UK). *N*,*N*′-Dicyclohexylcarbodiimide (DCC, 99%) was purchased from Acros Organics. 2,2′-Azobis(isobutyronitrile) (AIBN) and maleimido-monoamide DOTA (≥94%) were purchased from BDH and Macrocyclics™ (Dallas, TX, USA), respectively.

The MPC, OEGMA and GMA monomers were used without further purification. The DMA monomer was passed through a neutral alumina column before use to remove the inhibitor. LA was recrystallized 4–5 times from ethyl acetate prior to use. The solvents THF, DMF, chloroform and methanol were used as the mobile phase in GPC, whereas *n*-hexane and ethyl acetate were

used in column chromatography. Regenerated cellulose dialysis membranes with molecular weight cut-off (MWCO) of 1000 Da were purchased from Spectra/Por. The disulfide-based dimethacrylate (DSDMA) branching monomer was synthesized following a previously reported method.[64]

Synthesis of hydroxyl-functional RAFT CTA

CADB (3.00 g, 10.74 mmol) and 1,6-hexanediol (6.35 g, 53.70 mmol) were added in a round-bottom flask equipped with a magnetic stir bar. These reagents were dissolved in 75 mL $CHCl_3$ by stirring the contents of the flask for 15 min at 40 °C. The reaction catalysts DCC (2.44 g, 11.81 mmol) and DMAP (131 mg, 1.07 mmol) were dissolved in 15 mL $CHCl_3$ at ambient temperature. The catalyst mixture was subsequently added with a syringe to the round-bottom flask. The reaction mixture was stirred under reflux for 46 h and allowed to cool in the refrigerator overnight. After filtration, $CHCl_3$ was removed under reduced pressure using a rotary evaporator. The resulting oily residue was purified by column chromatography (silica gel/*n*-hexane–ethyl acetate 50 : 50). After removal of the solvents using a rotary evaporator and a vacuum oven, an oily liquid was obtained (1.82 g, 45% yield).

^{1}H NMR ($CDCl_3$): δ = 1.32–1.36 (m, 4H), 1.52 (m, 2H), 1.61 (m, 2H), 1.89 (s, 3H), 2.33–2.67 (m, 4H), 3.57 (t, 2H), 4.06 (t, 2H), 7.33–7.87 (m, 5H, aromatic).

^{13}C NMR ($CDCl_3$): δ = 24.2 (CH_3), 25.5 ($COOCH_2CH_2\mathit{CH_2}$), 25.9 ($\mathit{CH_2}COO$), 28.6 ($\mathit{CH_2}CH_2CH_2OH$), 30.0 ($COOCH_2\mathit{CH_2}$), 32.7 ($\mathit{CH_2}CH_2OH$), 33.6 ($\mathit{CH_2}CH_2COO$), 45.9 ($SCCH_2$), 62.7 ($\mathit{CH_2}CH_2OH$), 65.3 ($COO\mathit{CH_2}$), 118.7 (CN), 126.8, 128.7, 133.2, 144.6 (Ph), 171.8 (CO), 222.5 (CS). ESI-MS, m/z $(M + H)^+$ 380.

Synthesis of PLA-based diblock copolymers

Simultaneous ROP–RAFT polymerization. For a typical simultaneous ROP–RAFT polymerization targeting PLA_{30}–$PDMA_{30}$ block copolymer at 66.7% w/w solids, the protocol was as follows: LA (0.53 g, 3.65 mmol), DMA (0.62 mL, 0.57 g, 3.65 mmol), **1** (0.05 g, 0.12 mmol), AIBN (4.0 mg, 0.02 mmol), DMAP (0.06 g, 0.40 mmol) and 0.79 mL 1,2-dichloroethane were mixed together in a 5 mL round-bottom flask. The flask was equipped with a magnetic flea and sealed with a rubber septum. The reaction mixture was purged with nitrogen for 20 min before being placed in a preheated oil bath at 74 °C for 24 h.

The syntheses of other PLA–PDMA and PLA–POEGMA block copolymers were conducted following the same one-step protocol. Representative ^{1}H NMR spectra of diblock copolymers prepared by simultaneous ROP–RAFT processes are given in Fig. 1(c–d).

Two-step ROP–RAFT polymerization. A protocol for the synthesis of a PLA_{30}–$PMPC_{30}$ block copolymer is described. In a 10 mL round-bottom flask equipped with a magnetic stir bar and sealed with a rubber septum, LA (0.53 g, 3.65 mmol), **1** (0.05 g, 0.12 mmol), DMAP (0.06 g, 0.40 mmol) and 1.90 mL 1,2-dichloroethane were added, the mixture was purged with nitrogen for 20 min, and then heated at 74 °C for 24 h. The reaction mixture was cooled to 20 °C and a sample was withdrawn for ^{1}H NMR and GPC analysis. Subsequently, MPC (1.08 g, 3.65 mmol), ACVA (8.5 mg, 0.03 mmol) and 8.71 mL anhydrous ethanol were added. The reaction solution was placed in a preheated oil bath at 78 °C for 24 h.

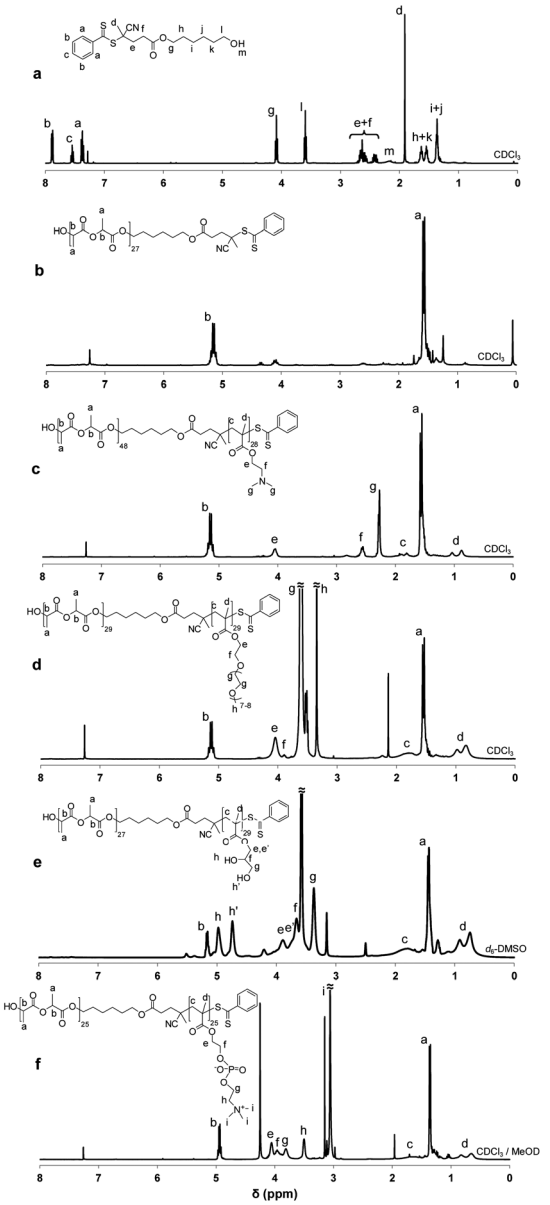


Fig. 1 ^{1}H NMR spectra of (a) ROP–RAFT dual agent **1**, (b) PLA_{27} macro-CTA, diblock copolymers by simultaneous (c) PLA_{48}–$PDMA_{28}$ **2** and (d) PLA_{29}–$POEGMA_{29}$ **2**, and by two-step (e) PLA_{27}–$PGMA_{29}$ **3a** and (f) PLA_{25}–$PMPC_{25}$ **3b** ROP–RAFT polymerization.

A similar two-step protocol was followed for the synthesis of a PLA_{200}–$PMPC_{30}$ block copolymer with a longer hydrophobic PLA block. The same method was applied for the preparation of PLA_{30}–$PGMA_{30}$ block copolymer and PLA_{30}–$PDMA_{30}$ block copolymer (for direct comparison with the one-step protocol). 1,2-Dichloroethane was used as a solvent for both polymerization steps. AIBN was the initiator for these two RAFT polymerizations.

All polymerizations (for both the simultaneous and the two-step protocols) were quenched by allowing the reaction solution to cool to 20 °C. A sample was removed from each reaction

Table 1 Summary of the monomer conversions, mean degrees of polymerization (DP), molecular weight data and intensity-average diameters of the diblock copolymers prepared by ROP–RAFT polymerization, see Scheme 1. Entries 1–7 were simultaneous ROP–RAFT polymerizations, whereas entries 8–11 were two-step ROP–RAFT polymerizations

			Conv.[b] (%)		DP_{Calcd}		DP_{NMR}		M_n (kDa)				
Entry[a]	M_{RAFT}	$[LA]_0 : [M_{RAFT}]_0 : [\mathbf{1}]_0$	LA	M_{RAFT}	LA	M_{RAFT}	LA	M_{RAFT}	Calcd.[c]	NMR	GPC	M_w/M_n[d]	d[e] (nm)
1	DMA	250 : 30 : 1	81	79	203	24	173	24	33.3	29.1	12.6	1.30	214
2	DMA	150 : 30 : 1	77	89	116	27	137	27	21.2	24.4	11.1	1.30	133
3	DMA	60 : 30 : 1	80	86	48	26	58	27	11.4	13.0	7.2	1.39	240
4	DMA	60 : 30 : 1	82	97	49	29	48	28	12.0	11.7	6.0	1.34	497
5	DMA	45 : 30 : 1	93	97	42	29	41	30	11.0	11.0	5.9	1.37	113
6	DMA	30 : 30 : 1	89	89	27	27	26	25	8.4	8.1	5.3	1.37	685
7	OEGMA	30 : 30 : 1	96	99	29	30	29	29	18.0	17.7	19.5	1.32	550
8	DMA	30 : 30 : 1	95	74	29	22	27	21	8.0	7.6	4.1	1.41	718
9	GMA	30 : 30 : 1	95	>99	29	30	27	29	9.1	8.9	42.3	1.27	338
10	MPC	200 : 30 : 1	92	79	184	24	184	23	34.0	33.7	70.5	1.35	1430
11	MPC	30 : 30 : 1	93	85	28	26	25	25	11.9	11.4	20.8	1.43	935

[a] *Polymerization conditions*: for simultaneous ROP–RAFT polymerization (entries 1–7) $[\mathbf{1}]_0 : [DMAP]_0 : [AIBN]_0 = 1 : 4 : 0.20$, 74 °C, 24 h, 55% w/w solids; for two-step ROP–RAFT polymerization (entries 8 and 9) $[\mathbf{1}]_0 : [DMAP]_0 : [AIBN]_0 = 1 : 4 : 0.20$, 74 °C, 24 h, 55% w/w solids; for two-step ROP–RAFT polymerization (entries 10 and 11) $[\mathbf{1}]_0 : [DMAP]_0 : [ACVA]_0 = 1 : 4 : 0.25$, 78 °C, 24 h, 20% w/w solids. [b] By ^{1}H NMR analysis ($CDCl_3$ for 1–7 and 8, d_6-DMSO for entry 9 and $CDCl_3$/MeOD 1 : 1 for entries 10 and 11). [c] $M_{n,Calcd} = MW_{LA} \times ([LA]_0/[\mathbf{1}]_0) \times (conv._{LA}) + (MW \text{ of } M_{RAFT}) \times ([M_{RAFT}]_0/[\mathbf{1}]_0) \times (conv. \text{ of } M_{RAFT}) + MW_1$. [d] By GPC (eluent THF for entries 1–7 and 8, DMF for entry 9, and $CHCl_3/CH_3OH$ 3 : 1 for entries 10 and 11). [e] Diameter d is the sphere-equivalent intensity-average diameter measured by DLS in water after a solvent switch protocol.

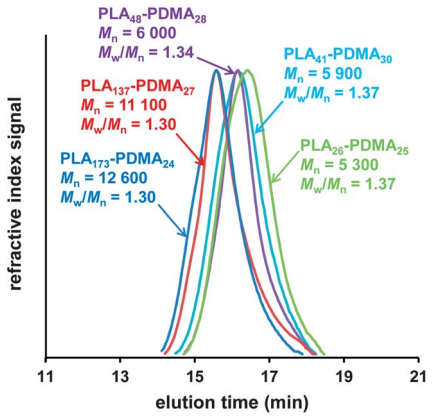


Fig. 2 THF GPC curves (*vs.* poly(methyl methacrylate) standards) obtained for PLA_x–$PDMA_y$ linear diblock copolymers synthesized *via* simultaneous ROP–RAFT polymerization in 1,2-dichloroethane at 74 °C, see Table 1 (entries 1, 2, 4, 5, 6).

solution for ^{1}H NMR and GPC analysis. The final copolymers were purified by dialysis against acetone using membranes with a MWCO of 1000. For PLA–PGMA and PLA–PMPC block copolymers, this was followed by dialysis in methanol. The solvent was evaporated using a rotary evaporator. The copolymers were dried in a vacuum oven for 48 h. The resulting block copolymers were characterized by ^{1}H NMR spectroscopy (Table 1 and Fig. 1) and GPC (Table 1, Fig. 2 and 3).

Synthesis of branched PLA-based diblock copolymers

Simultaneous ROP–RAFT polymerization. The protocol for the simultaneous ROP–RAFT synthetic process yielding the PLA_{30}–P(OEGMA$_{30}$-*stat*-DSDMA$_1$) at 55% w/w solids, is described: LA (0.20 g, 1.37 mmol), OEGMA (0.62 g, 1.37 mmol), DSDMA (0.01 g, 0.05 mmol), **1** (0.02 g, 0.05 mmol), AIBN (1.5 mg, 0.01 mmol), DMAP (0.02 g, 0.18 mmol) and 0.57 mL 1,2-dichloroethane were added in a 5 mL round-bottom flask.

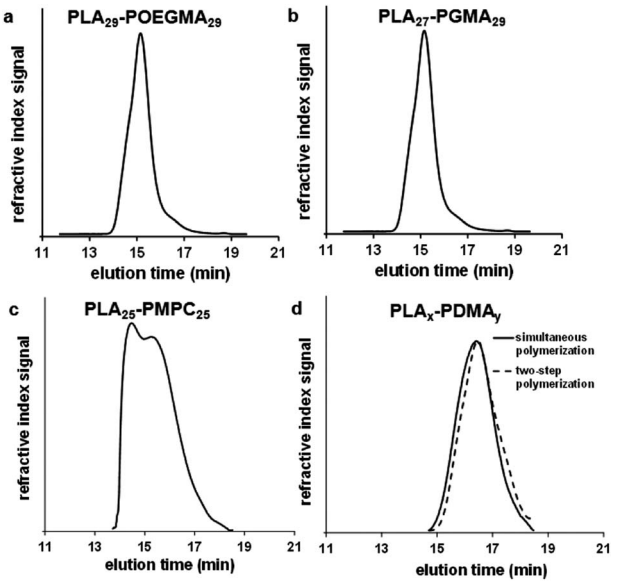


Fig. 3 GPC curves (*vs.* poly(methyl methacrylate) standards) obtained for (a) PLA_{29}–$POEGMA_{29}$ linear diblock copolymer (THF eluent) synthesized *via* simultaneous ROP–RAFT polymerization in 1,2-dichloroethane at 74 °C, (b) PLA_{27}–$PGMA_{29}$ linear diblock copolymer (DMF eluent) synthesized *via* two-step ROP–RAFT polymerization in 1,2-dichloroethane at 74 °C, (c) PLA_{25}–$PMPC_{25}$ linear diblock copolymer ($CHCl_3/CH_3OH$ 3 : 1 v/v eluent) prepared by two-step ROP–RAFT polymerization (ROP of LA in 1,2-dichloroethane at 74 °C followed by RAFT polymerization of MPC in ethanol at 78 °C) and (d) comparison of $PLDMA_{25}$ and PLA_{27}–$PDMA_{21}$ prepared by simultaneous and two-step ROP–RAFT polymerizations, respectively, in 1,2-dichloroethane at 74 °C.

The flask was equipped with a magnetic stir bar and sealed with a rubber septum. The reaction mixture was purged with nitrogen for 20 min and placed in an oil bath at 74 °C for 24 h. A viscous (gel-like) copolymer solution was obtained. The copolymer was characterized by ^{1}H NMR ($CDCl_3$) and GPC (DMF, $M_n = 56\,900$ g mol^{-1}, $M_w = 134\,000$ g mol^{-1}, $M_w/M_n = 2.36$), see Fig. 4.

The branched PLA_{30}–P(DMA_{30}-*stat*-$DSDMA_1$) block copolymer was synthesized using a similar protocol. This resulted in the formation of a gel.

Disulfide cleavage of branched PLA_{30}–P($OEGMA_{30}$-*stat*-$DSDMA_1$) diblock copolymer, functionalization of the resulting PLA_{30}–P($OEGMA_{30}$-*stat*-$TEMA_2$) block copolymer with divinyl sulfone and its subsequent conjugation with L-glutathione

500 mg (275 mg solids content, 30.6 μmol disulfide bonds) of the branched PLA_{30}–P($OEGMA_{30}$-*stat*-$DSDMA_1$) polymer were added in a 50 mL round-bottom flask. Subsequently, 20 mL DMF (1.4% w/v) was added to the flask to dissolve the reagents. The flask was equipped with a magnetic stir bar and sealed with a rubber septum. The mixture was purged with nitrogen gas for 20 min and placed in an oil bath at 30 °C. A solution of Bu_3P (91.9 μmol, 18.6 mg, 3.0 eq. relative to disulfide bonds) and Et_3N (64.3 μmol, 6.5 mg, 2.1 eq. relative to disulfide bonds) in 5 mL DMF was added under a nitrogen atmosphere *via* syringe to the round-bottom flask. This resulted in disulfide cleavage, allowing the conversion of 1 eq. of DSDMA to 2 eq. of TEMA. The reaction was left in an oil bath for 2 h at 30 °C to afford a thiol-functionalized linear PLA_{30}–P($OEGMA_{30}$-*stat*-$TEMA_2$) block copolymer solution. A sample was extracted from the reaction flask for DMF GPC analysis ($M_n = 38\,800$ g mol^{-1}, $M_w = 53\,100$ g mol^{-1}, $M_w/M_n = 1.37$). A degassed solution of divinyl sulfone (0.92 mmol, 108.6 mg, 15 eq. relative to the thiol –SH group) in 5 mL DMF was added to the block copolymer. The resulting mixture was stirred at 30 °C for 15 h to convert the TEMA monomer units into VSTEMA monomer units. The final solution was dialyzed (MWCO 1000 Da) against acetone (5 times/3 days). The solvent was removed by rotary evaporator, followed by vacuum oven drying. The dried PLA_{30}–P($OEGMA_{30}$-*stat*-$VSTEMA_2$) was characterized by DMF GPC ($M_n = 37\,800$ g mol^{-1}, $M_w = 52\,800$ g mol^{-1}, $M_w/M_n = 1.40$) and ^{1}H NMR (Fig. 4).

202 mg of the above dried vinyl sulfone-functionalized block copolymer (218.4 mmol vinyl sulfone, assuming 2 vinyl sulfones per polymer chain) were placed in a 10 mL glass vial equipped with a magnetic stir bar and sealed with a rubber septum. A mixture of 7.0 mg Glu (229.3 mmol Glu or free thiol group, 1.05 eq. relative to vinyl sulfone), 0.6 mg TCEP (0.1 eq. relative to vinyl sulfone) and 4 mL water was purged with nitrogen gas for 20 min. This solution was then added to the vial containing the copolymer. The resulting mixture was purged with nitrogen for another 10 min. The reaction was allowed to proceed a 20 °C with stirring for 4 h. The final copolymer solution was dialyzed (MWCO 1000 Da) against water (6 times/1 day). The purified PLA_{30}–P($OEGMA_{30}$-*stat*-$GluVSTEMA_2$) was freeze-dried. The dried copolymer was dissolved in the appropriate solvent and characterized by DMF GPC ($M_n = 23\,600$ g mol^{-1}, $M_w = 34\,100$ g mol^{-1}, $M_w/M_n = 1.45$) and ^{1}H NMR (Fig. 4 in $CDCl_3$ and Fig. S2† in D_2O).

Disulfide cleavage of the branched PLA_{30}–P(DMA_{30}-*stat*-$DSDMA_1$) diblock copolymer, functionalization of the resulting PLA_{30}–P(DMA_{30}-*stat*-$TEMA_2$) block copolymer with maleimido-monoamide-DOTA and subsequent In conjugation

182 mg (100 mg solids content, 10.3 μmol disulfide bonds) of the PLA_{30}–P(DMA_{30}-*stat*-$DSDMA_1$) gel was placed in a 14 mL glass vial containing 5 mL DMF (2.0% w/v). The flask was equipped with a magnetic stir bar and sealed with a rubber septum. The mixture was placed in an oil bath at 30 °C after purging with nitrogen for 20 min. A solution of Bu_3P (30.9 μmol, 6.3 mg, 3.0 eq. relative to disulfide bonds) and Et_3N (21.6 μmol, 2.2 mg, 2.1 eq. relative to disulfide bonds) in 1 mL DMF was prepared and added under nitrogen atmosphere to the glass vial using a syringe. The reaction mixture was left at 30 °C for 2 h, resulting in cleavage of the disulfide bonds. With this reaction the gel network was converted to a thiol-functionalized linear PLA_{30}–P(DMA_{30}-*stat*-$TEMA_2$) block copolymer solution. Maleimido-monoamide-DOTA was reacted with the TEMA residues to produce DOTA-functionalized TEMA (DOTATEMA) residues. Specifically, a degassed solution of maleimido-monoamide-DOTA (21.6 μmol, 17.0 mg, 2.1 eq. relative to disulfide bonds) was added. The reaction was allowed to proceed at 30 °C overnight. Then $InCl_3$ (4.6 mg, 20.6 μmol, 2.0 eq. relative to disulfide bonds) were added and the mixture was stirred at 90 °C for 1 h. The solution was dialyzed against acetone (MWCO 1000 Da/5 times/3 days) and the solvent was removed using rotary evaporator. The resulting In-conjugated PLA_{30}–P(DMA_{30}-*stat*-InDO-$TATEMA_2$) block copolymer was dried in a vacuum oven overnight and characterized by ICP-AES (1.16 In atoms per copolymer chain).

Self-assembly of amphiphilic block copolymers in dilute aqueous solution

Self-assembly of the block copolymers was carried out using the solvent switch method. Briefly, PLA–PDMA and PLA–POEGMA block copolymers were dissolved in acetone to give 50 mg per mL of copolymer solutions. 4.8 mL deionized water was added drop by drop in 200 μL of the above solutions under stirring giving copolymer solutions with a final concentration of 2.5 mg mL^{-1}. Similarly, an acetone–methanol mixture (3 : 1 v/v) was used to solubilize the PLA–PGMA block copolymer. These solutions were stirred vigorously at room temperature for 5 h, allowing acetone evaporation. For more efficient solvent evaporation the solution was left on a rotary evaporator for 1 min. Following a similar method, the PLA_x–$PMPC_y$ block copolymers were dissolved in a 3 : 1 v/v chloroform–methanol mixture. This was followed by addition of water and stirring for 2 h to allow solvent evaporation. Dialysis was performed against deionized water for 4 h (5 times) to remove trace organic solvents. Finally, all copolymer solutions were stored at 4 °C overnight to avoid copolymer degradation.

Polymer characterization

PLA–PDMA and PLA–POEGMA block copolymers were assessed using a GPC system purchased from Polymer Laboratories at 30 °C. The system comprised two PL Gel 5 μm (7.5 × 300 mm) Mixed-C columns in series with a guard column. These were connected with a WellChrom K-2301 refractive index (RI) detector operating at 950 ± 30 nm. THF containing 2% v/v triethylamine and 0.05 wt/v % of butylhydroxytoluene (BHT) (flow rate 1 mL min^{-1}) was used as an eluent.

PLA–PMPC block copolymers were assessed using a Hewlett Packard HP1090 Liquid Chromatograph. Two PL Gel 5 μm Mixed-C columns in series with a guard column at 40 °C and a Gilson Model 131 RI detector were used. The eluent was a 3 : 1 v/v chloroform–methanol mixture with 2 mM lithium bromide (LiBr, flow rate of 1.0 mL min^{-1}). Toluene (2 μL) was added to the sample as a flow rate marker.

Ten near-monodisperse linear poly(methyl methacrylate) (PMMA) standards (ranging from 1280 g mol^{-1} to 330 000 g mol^{-1}) were purchased from Polymer Laboratories (UK). These were used to calibrate the above two RI detectors. For both GPC systems, data analysis was carried out using Cirrus™ GPC Software supplied by the manufacturer.

The MWDs of the PLA–PGMA block copolymer, the branched PLA_{30}–P($OEGMA_{30}$-*stat*-$DSDMA_1$) block copolymer, the disulfide cleaved PLA_{30}–P($OEGMA_{30}$-*stat*-$TEMA_2$) linear block copolymer and the functionalized linear block copolymers PLA_{30}–P($OEGMA_{30}$-*stat*-$VSTEMA_2$) and PLA_{30}–P($OEGMA_{30}$-*stat*-$GluVSTEMA_2$) were assessed by DMF GPC. This system comprised two Polymer Laboratories PL gel 5 μm mixed C columns and one PL polar gel 5 μm guard column. These were arranged in series and maintained at 60 °C, followed by a Varian 390 LC RI detector. The DMF eluent containing 10 mM LiBr was kept at a flow rate of 1.0 mL min^{-1}. Ten near monodisperse PMMA standards with MWs ranging from 625 g mol^{-1} to 618 000 g mol^{-1} were used for calibration.

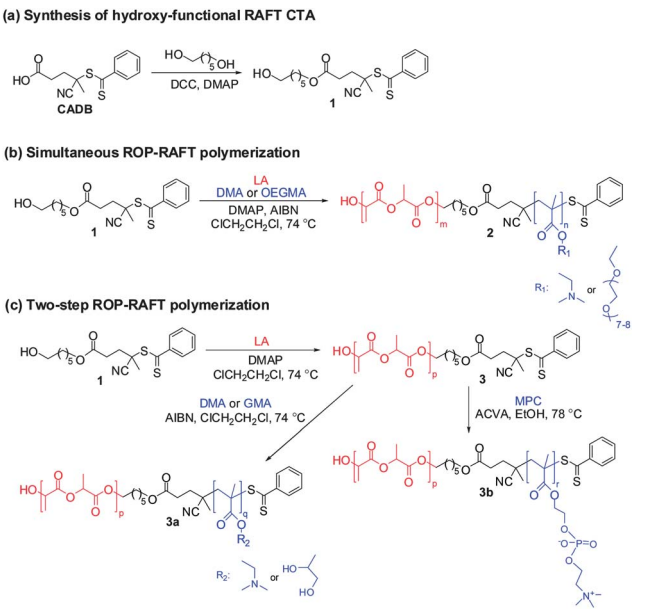
^1H (Fig. 1a) and ^{13}C NMR studies of the ROP–RAFT dual agent were performed in $CDCl_3$. ^1H NMR spectra of the PLA macroCTAs and the copolymers were acquired in the appropriate solvent for each block copolymer (Fig. 1b–f). A 250 or 400 MHz Bruker spectrometer was used for NMR analysis. The experimental DP values (DP_{NMR}) of the PLA polymer block were obtained by comparing the integrated signal intensities of the aromatic RAFT end-group at 7.10–7.80 ppm with those of the –C*H*– protons of PLA at 5.14–5.24 ppm in d_6-DMSO. The experimental DP values (DP_{NMR}) of the RAFT-synthesized block were observed by comparing the integrated intensities of its characteristic protons with those of the methine protons from PLA. In more detail, for the calculations, the methyl protons of PDMA at 2.22–2.33 ppm in $CDCl_3$ (Fig. 1c), the –COOCH_2 protons of POEGMA block at 4.04 ppm in $CDCl_3$ (Fig. 1d), the –CH_2OH protons of PGMA at 3.38 ppm in d_6-DMSO (Fig. 1e), and the methylene protons of the PMPC polymer block (Fig. 1f, protons e, f and g) at 3.65–4.14 ppm were used for the RAFT-synthesized block. For the PLA block, the methine protons of PLA at 5.07–5.24 ppm in $CDCl_3$, at 5.13–5.21 ppm in d_6-DMSO or 4.84–5.01 ppm in 1 : 1 $CDCl_3$: CD_3OD were taken into account. All the results are presented in Table 1.

DLS experiments were performed using a Zetasizer Nano-ZS (Malvern Instruments, UK). Aqueous copolymer solutions (0.1% w/v) were analyzed using disposable cuvettes and data were averaged over three consecutive runs.

TEM imaging of copolymer samples was performed using a FEI Tecnai G2 Spirit TEM 120 kV instrument equipped with an Orius SC1000 camera. The TEM samples were prepared using in-house carbon-coated copper grids. Grids were plasma glow-discharged for 45 s to create a hydrophilic surface. Each aqueous copolymer solution (0.1% w/w) was placed onto a freshly glow-discharged grid for 30 s. The grid was then blotted with a filter paper to remove excess solution. For positive staining of the deposited nanoparticles, a uranyl formate solution (0.75 w/v %) was used. This was placed using a micropipette on the sample-loaded grid for 20 s. The grid was then blotted with filter paper and dried using a vacuum hose.

Results and discussion

In this work, amphiphilic diblock copolymers were synthesized consisting of a hydrolyzable aliphatic polyester block (PLA) and a methacrylic polymer block consisting of PDMA, POEGMA, PGMA or PMPC. The preparation of these copolymers is illustrated in Scheme 1. A combined ROP–RAFT agent **1** containing a



Scheme 1 (a) Synthesis of hydroxyl-functional RAFT CTA for use as a ROP–RAFT dual agent in polymerizations. (b) Simultaneous ROP–RAFT copolymerization of (3*S*)-*cis*-3,6-dimethyl-1,4-dioxane-2,5-dione (L-lactide, LA) with 2-(dimethylamino)ethyl methacrylate (DMA) or oligo(ethylene glycol) methacrylate (OEGMA) at 74 °C in 1,2-dichloroethane to produce amphiphilic polylactide–methacrylic block copolymers **2**. (c) Two-step ROP–RAFT polymerization; ROP of LA at 74 °C in 1,2-dichloroethane to synthesize PLA macro-CTAs that are subsequently used in the RAFT polymerization of DMA and glycerol monomethacrylate (GMA) at 74 °C in 1,2-dichloroethane or 2-(methacryloyloxy)ethyl phosphorylcholine (PMPC) at 78 °C in anhydrous ethanol for the preparation of amphiphilic polylactide–methacrylic block copolymers **3a** and **3b**, respectively.

hydroxyl group as the initiating site for ROP and a dithiobenzoate functionality for the RAFT polymerization was utilized. This CTA is the monoester product of the reaction between CADB and 1,6-hexanediol (Scheme 1a). The esterification was conducted in chloroform under reflux for 48 h using DCC (1.1 eq.) and DMAP (0.1 eq.) as catalyst. To ensure a monofunctional product, excess diol (5.0 eq.) was used. The ROP–RAFT dual agent **1** was obtained in 45% yield. Its chemical structure and high purity were confirmed by ^{1}H NMR spectroscopy (Fig. 1a), ^{13}C NMR and electrospray ionization mass spectroscopy (ESI-MS) analysis.

Subsequently, the ROP–RAFT dual agent **1** was used in the synthesis of block copolymers using both simultaneous one-step (Scheme 1b) and two-step (Scheme 1c) protocols. LA was used as the ROP monomer for the formation of all block copolymers (**2**, **3a** and **3b**). The resulting PLA homopolymer **3** was used as a macro-CTA in the second polymerization step (for the preparation of **3a** or **3b**). Various LA/**1** molar ratios were used for the preparation of a PLA$_x$–PDMA$_y$ block copolymer **2** *via* simultaneous polymerization (Scheme 1b). An LA/**1** molar ratio of 30 was also utilized for the synthesis of a PLA–POEGMA block copolymer **2** by the same simultaneous polymerization protocol (Scheme 1b). The same molar ratio was also used for PLA–PDMA **3a**, PLA–PGMA **3a** and PLA–PMPC **3b** block copolymer syntheses by two-step polymerization (Scheme 1c). In all cases, the molar ratio of the RAFT monomer (DMA, OEGMA, GMA or PMPC) to **1** was kept constant at 30.

More specifically, for the synthesis of diblock copolymers by simultaneous ROP–RAFT polymerization (see Scheme 1b) either DMA or OEGMA was used as the RAFT monomer and 1,2-dichloroethane (bp 84 °C) as a solvent (55% w/w solids). A two-step process was required for the preparation of the diblock copolymer containing glycerol monomethacrylate (GMA) *i.e.* PLA–PGMA **3a**. This was because the hydroxyl groups in the GMA monomer structure are capable of initiating unwanted ROP polymerization. Following the same two-step protocol (Scheme 1c), a PLA–PDMA block copolymer **3a** was synthesized for comparison with its one-step synthetic process (Scheme 1b). Both steps were performed in 1,2-dichloroethane (36.0% and 55% w/w solids for first and second step, respectively). A two-step protocol was also required for the synthesis of the PLA–PMPC block copolymers. Such copolymers cannot be synthesized by simultaneous ROP–RAFT polymerization due to the insolubility of the MPC monomer in any suitable aprotic solvent for ROP. First, ROP of LA produced a PLA homopolymer **3** ($[LA]_0 : [\mathbf{1}]_0 = 30$ or 200) in 1,2-dichloroethane (36.0% w/w solids). Subsequently, MPC was employed as the RAFT monomer in the second step. Anhydrous ethanol (b.p. 78 °C, 20% w/w solids) was used for the synthesis of the PLA–PMPC block copolymers **3b** (second step, Scheme 1c). Ethanol is a good solvent for both MPC monomer and PMPC. DMAP was selected as the catalyst for the ROP of LA instead of the more commonly-used catalyst stannous octoate (Sn(Oct)$_2$) for this polymerization technique.[6,7,16,17,46] DMAP can be used at lower temperatures,[46] making it compatible with the RAFT polymerization conditions.[24] 2,2′-Azobis(isobutyronitrile) (AIBN) was used as the thermal initiator for RAFT polymerization. This initiator was utilized in both the one-step process **2** and the two-step process **3a** ($[\mathbf{1}]_0 : [AIBN]_0 = 1 : 0.20$). ACVA was the initiator in the two-step process **3b** ($[\mathbf{1}]_0 : [ACVA]_0 = 1 : 0.25$).

All reactions conducted in 1,2-dichloroethane (Scheme 1b and c polymers **3** and **3a**) were performed at 74 °C for 24 h. The PLA–PMPC block copolymer synthesis **3b** (Scheme 1c) was conducted in ethanol at 78 °C for 24 h. The monomer conversions were determined by ^{1}H NMR analysis. Conversions were calculated based on the resonance intensities of the remaining unreacted monomers: LA at 5.03–5.10 ppm in $CDCl_3$ (methine proton). DMA at 5.55 and 6.09 ppm, OEGMA at 5.52 ppm and 6.08 ppm in $CDCl_3$, GMA at 5.66 and 6.05 ppm in d_6-DMSO and MPC at 5.38 and 5.90 ppm in $CDCl_3$/CD_3OD (for four methacrylic protons for each monomer). As shown in Table 1, relatively high conversions were achieved for all monomers, ranging from 77% to 96% for the LA monomer and between 74% and 99% for the methacrylic monomers (DMA, OEGMA, GMA and MPC). The one-step synthesis of the PLA–POEGMA block copolymer gave the highest monomer conversions; 96% for LA and 99% for OEGMA (PLA_{29}–$POEGMA_{29}$, Table 1, entry 7). The corresponding monomer conversions for other block copolymers prepared by the one-step protocol using the same feed ratios ($[LA]_0 : [\text{RAFT monomer}]_0 : [\mathbf{1}]_0 = 30 : 30 : 1$) were 95% and 74% for PLA_{27}–$PDMA_{21}$ (Table 1, entry 8) and, 95% and >99% for PLA_{27}–$PGMA_{29}$ (Table 1, entry 9). For the PLA–PMPC block copolymer prepared by two-step polymerization (PLA_{25}–$PMPC_{25}$, Table 1, entry 11) the resulting conversions were 93% for LA (first step) and 85% for MPC (second step). In the one-step synthesis of PLA$_x$–PDMA$_y$ block copolymers (Table 1, entries 1–6), the conversions of both LA and DMA monomers were not significantly affected by changing the feed ratio of LA monomer to **1** ($[LA]_0 : [1]_0$ from 30 for entry 6 to 250 for entry 1). These values ranged from 77% to 93% for the LA monomer and between 79% and 97% for the DMA monomer.

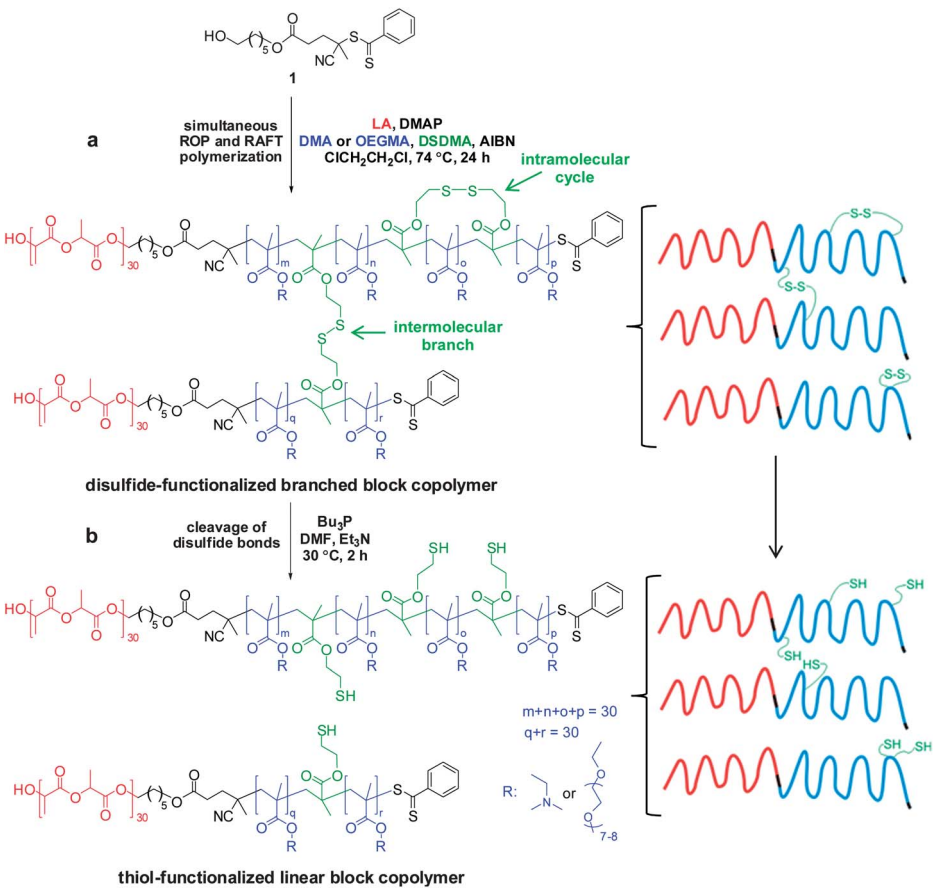
All diblock copolymers were purified by dialysis against acetone. This was followed by methanol dialysis in the case of PLA–PGMA and PLA–PMPC block copolymers. This second dialysis step was essential for removal of any unreacted GMA or MPC monomer respectively, which are insoluble in acetone. After solvent evaporation, the dried block copolymers were characterized by ^{1}H NMR spectroscopy and gel permeation chromatography (GPC). The results are presented in Table 1, Fig. 1, 2 and 3.

The degrees of polymerization (DPs) calculated by ^{1}H NMR (DP_{NMR}) were found to be in agreement with the theoretical values (DP_{Calcd}) calculated from the monomer feed ratios. These results suggest the well-controlled formation of block copolymers both for the one- and two-step protocols, except in the case where MPC was used as a monomer due to the partial insolubility of the PLA macro-CTA in ethanol, which was the polymerization solvent used for this RAFT synthesis. The molecular weight distributions (MWDs) of these copolymers were relatively narrow (Fig. 2 and 3): polydispersities ranged between 1.30 and 1.43 (see Table 1). The two PLA_{30}–$PDMA_{30}$ diblock copolymers had similar M_n (NMR) values of 8.1 kDa and 7.6 kDa when prepared by either the one-step (Table 1, entry 6) or two-step (Table 1, entry 8) protocols, respectively.

The MWD for the PLA_{30}–$PDMA_{30}$ diblock copolymer prepared by one-step polymerization was slightly narrower than that obtained *via* the two-step protocol (Fig. 3d). Polydispersities of 1.37 and 1.41 were obtained for the one-step and two-step protocols respectively (Table 1, entries 6 and 8). Thus using the more convenient protocol does not compromise the quality of the block copolymer. GPC analysis of PLA-based block copolymers using a refractive index detector is challenging because of the relatively low refractive index increment (d*n*/d*c*) of the aliphatic polyester component in common GPC eluents. For example, the d*n*/d*c* of PLA is 0.048 mL g^{-1} (ref. 70) in THF. This value is significantly lower than that for PMMA calibration standards (0.087 mL g^{-1} (ref. 71) in THF), which leads to inaccuracies in the GPC characterization of PLA-based block copolymers, particularly in the low molecular weight range.

Simultaneous ROP–RAFT polymerization using **1** was also utilized for the synthesis of PLA-based branched block copolymers. Here, either OEGMA or DMA was used as the RAFT monomer and DSDMA was selected as the cleavable cross-linker. A molar ratio of $[LA]_0 : [RAFT\ monomer]_0 : [DSDMA]_0 : [\mathbf{1}]_0 = 30 : 30 : 1 : 1$ was used to form a block copolymer with a linear PLA block and a branched statistical methacrylic block comprising OEGMA (or DMA) and DSDMA.

This synthetic route is presented in Scheme 2a. As shown in Fig. 4a, DMF GPC analysis (*vs.* PMMA calibration standards) of this disulfide-containing block copolymer indicated a relatively broad MWD ($M_w/M_n = 2.36$). This confirmed that the DSDMA comonomer had reacted not only intramolecularly but also intermolecularly,[63,65] resulting in branching. Cleavage of the disulfide bonds (Scheme 2b) using excess Bu_3P led to a much narrower MWD (Fig. 4a). The M_w/M_n was reduced from 2.36 to 1.37 for the resulting thiol-functionalized linear block copolymer PLA_{30}–$P(OEGMA_{30}$-*stat*-$TEMA_2)$ (where TEMA denotes 2-thioethyl methacrylate). Vinyl sulfone was reacted with the TEMA residues to afford 2-(2-(vinylsulfonyl)ethylthio)ethyl methacrylate (VSTEMA) units (Scheme 3a). For this thiol–ene reaction, a large excess of DVS (15 eq. relative to the thiol –SH group of TEMA) was used to ensure that only one of the DVS double bonds reacts with the thiol group. Thus possible inter- or intra-molecular cross-linking was avoided. The second double bond of the DVS thus remained unreacted and was utilized for further conjugation with thiol-containing



Scheme 2 Preparation of thiol-functionalized block copolymers. (a) Synthesis of a disulfide-functionalized branched block copolymer by simultaneous ROP of LA and RAFT statistical copolymerization of OEGMA or DMA with DSDMA using a dual ROP–RAFT reagent **1** at 55% w/w solids, at 74 °C for 24 h. Polymerization conditions: $[LA]_0 : [R]_0 : [DSDMA]_0 : [ROP–RAFT\ reagent]_0$ relative molar ratios 30 : 30 : 1 : 1. (b) Reductive cleavage of the disulfide bonds in the methacrylic block using Bu_3P in DMF at 30 °C for 2 h, results in the formation of linear statistical block copolymer containing thiol groups that can be used for further functionalization. *Reaction conditions*: $[Bu_3P]_0 : [Et_3N]_0 : [disulfide\ bond]_0$ relative molar ratios 3.0 : 2.1 : 1.

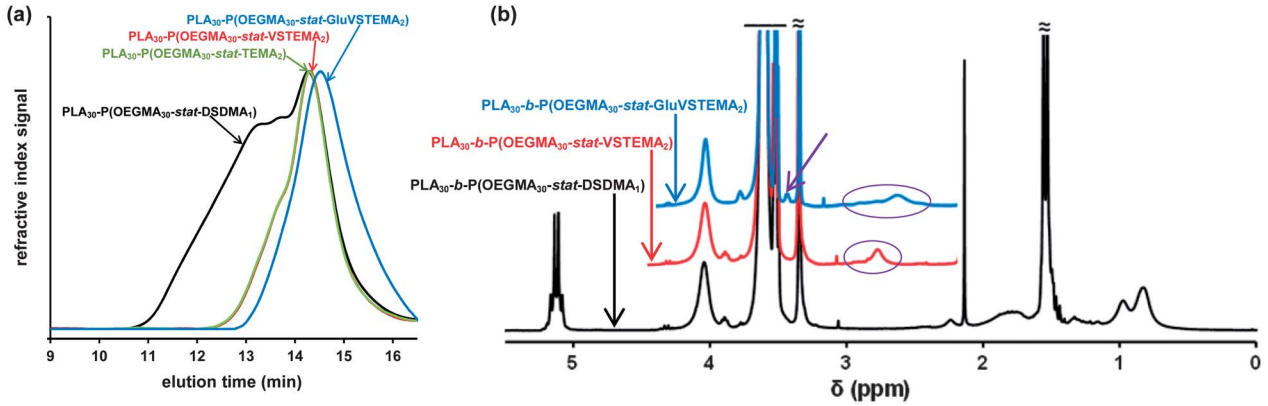
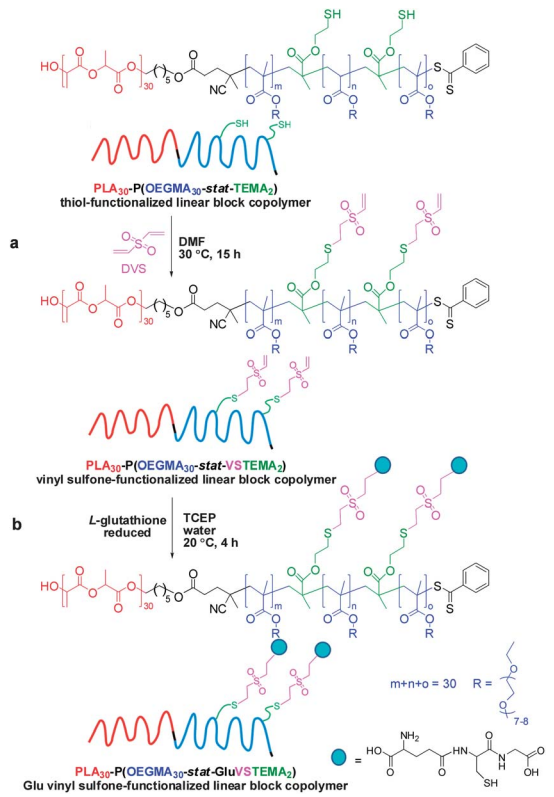


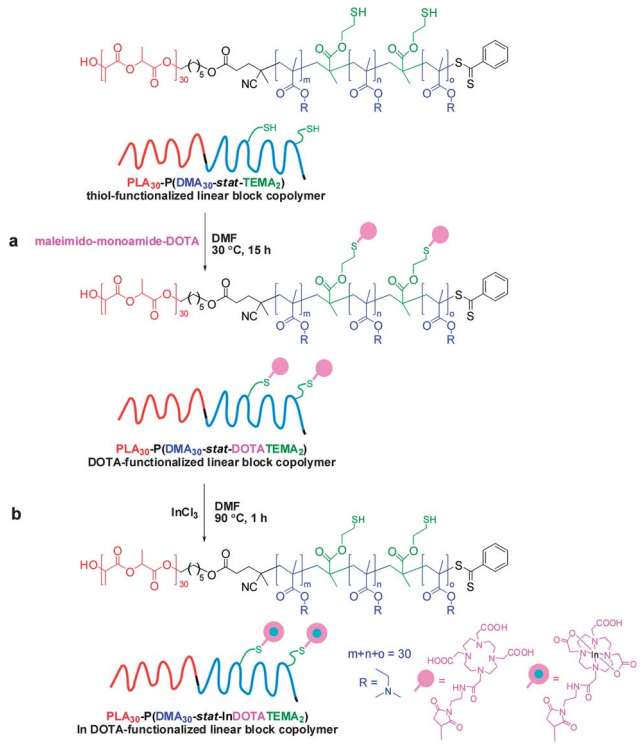
Fig. 4 (a) DMF GPC curves *vs.* poly(methyl methacrylate) standards and (b) ^{1}H NMR spectra (right; recorded in $CDCl_3$) obtained for the PLA_{30}–$P(OEGMA_{30}$-*stat*-$DSDMA_1)$ branched block copolymer, the PLA_{30}-*b*-$P(OEGMA_{30}$-*stat*-$TEMA_2)$ linear block copolymer obtained after disulfide cleavage using tributyl phosphine, the PLA_{30}–$P(OEGMA_{30}$-*stat*-$VSTEMA_2)$ linear block copolymer after functionalization with divinyl sulfone and the PLA_{30}-*b*-$P(OEGMA_{30}$-*stat*-$GluVSTEMA_2)$ after L-glutathione (Glu) conjugation (LA = lactide; OEGMA = oligo(ethylene glycol) methacrylate); DSDMA = disulfide-based dimethacrylate; TEMA = 2-thioethylmethacrylate; VSTEMA = 2-(2-(vinylsulfonyl)ethylthio)ethyl methacrylate, GluVSTEMA = glutathione conjugated 2-(2-(vinylsulfonyl)ethylthio)ethyl methacrylate).



Scheme 3 Preparation of a L-glutathione-conjugated linear block copolymer, PLA_{30}–$P(OEGMA_{30}$-*stat*-$GluVSTEMA_2)$. (a) Synthesis of a vinyl sulfone-functionalized linear block copolymer PLA_{30}–$P(OEGMA_{30}$-*stat*-$VSTEMA_2)$ by *in situ* conjugation of excess divinyl sulfone to the thiol-functionalized linear block copolymer, PLA_{30}–$P(OEGMA_{30}$-*stat*-$TEMA_2)$. *Reaction conditions*: DMF, 30 °C, 15 h, $[DVS]_0$: $[thiol]_0$ relative molar ratio 15 : 1. (b) Conjugation of glutathione to the vinyl sulfone-functionalized linear block copolymer PLA_{30}–$P(OEGMA_{30}$-*stat*-$VSTEMA_2)$. *Reaction conditions*: water, room temperature, 4 h, $[TCEP]_0$: $[thiol]_0$ molar ratio = 0.1.

molecules.[72] The success of the reaction for the synthesis of the vinyl sulfone-functionalized amphiphilic block copolymer, PLA_{30}–$P(OEGMA_{30}$-*stat*-$VSTEMA_2)$, was confirmed by ^{1}H NMR studies (see Fig. 4b and Fig. S1, ESI†). Specifically, in the ^{1}H NMR ($CDCl_3$) spectrum (Fig. S1†) the signal at 6.0–7.0 ppm is due to the pendant vinylsulfone protons. The signal at 2.75 ppm is assigned to the $-OCH_2CH_2SCH_2-$ protons. DMF GPC analysis of this copolymer indicates an M_w/M_n of 1.40, compared to 1.37 for the PLA_{30}–$P(OEGMA_{30}$-*stat*-$TEMA_2)$. This suggests that minimal branching occurred during RAFT copolymerization in this case. In principle, PLA_{30}–$P(OEGMA_{30}$-*stat*-$VSTEMA_2)$ can be reacted with thiol-functional oligopeptides, which can be useful for biological applications. This concept was tested in a model reaction using Glu (Scheme 3b). The conjugation reaction was performed under mild conditions (20 °C, 4 h, water). A Glu/VSTEMA molar ratio of 1.05 and a small amount of TCEP catalyst ($[TCEP]_0 : [thiol]_0 = 0.10$) were used. The resulting PLA_{30}–$P(OEGMA_{30}$-*stat*-$GluVSTEMA_2)$ block copolymer had a similar M_n/M_w value (1.45 *vs.* 1.40) with the PLA_{30}–$P(OEGMA_{30}$-*stat*-$VSTEMA_2)$ precursor. However, the former copolymer had a much lower M_n (23 600 g mol^{-1} *vs.* 37 800 g mol^{-1}) (DMF GPC, Fig. 4a) than the latter copolymer. This is attributed to partial hydrolysis of the PLA block in water during the conjugation reaction and purification by dialysis. Successful Glu conjugation was confirmed by ^{1}H NMR ($CDCl_3$) since the new signal at 3.51 ppm is assigned to the $-CH_2CH(NH_2)COOH$ proton. Also, in Fig. S2b (ESI†) the same peak was obtained at 3.48 ppm when D_2O was used as an NMR solvent.

A gel network was obtained when DMA was used as a comonomer for the synthesis of a disulfide-containing branched amphiphilic block copolymer, PLA_{30}–$P(DMA_{30}$-*stat*-$DSDMA_1)$ (Scheme 2a). Thus characterization of this precursor copolymer using GPC or ^{1}H NMR was not feasible. Gel formation indicates a significantly higher degree of branching than for the analogous reaction using OEGMA instead of DMA. A viscous solution rather than a gel was obtained from the



Scheme 4 Preparation of an In-conjugated linear block copolymer, PLA_{30}–$P(DMA_{30}$-*stat*-$InDOTATEMA_2)$. (a) Synthesis of a DOTA-functionalized linear block copolymer, PLA_{30}–$P(DMA_{30}$-*stat*-$DOTATEMA_2)$, by *in situ* conjugation of maleimido-monoamide-DOTA to the thiol-functionalized linear block copolymer, PLA_{30}–$P(DMA_{30}$-*stat*-$TEMA_2)$. *Reaction conditions*: DMF, 30 °C, 15 h, maleimido-monoamide-DOTA (2.1 eq. relative to disulfide bonds). (b) Conjugation of indium to the PLA_{30}–$P(DMA_{30}$-*stat*-$DOTATEMA_2)$ block copolymer. *Reaction conditions*: DMF, 90 °C, 1 h, $InCl_3$ (2.0 eq. relative to disulfide bonds).

synthesis of the PLA_{30}–$P(OEGMA_{30}$-*stat*-$DSDMA_1)$ branched block copolymer. Presumably, the sterically congested nature of the OEGMA residues (*vs.* DMA residues) hinders intermolecular cross-linking. Subsequently, the disulfide bonds of the PLA_{30}–$P(DMA_{30}$-*stat*-$DSDMA_1)$ branched block copolymer were reduced using the same protocol as described above for the OEGMA-based branched block copolymer (Scheme 2b). This resulted in the formation of a thiol-functionalized PLA_{30}–$P(DMA_{30}$-*stat*-$TEMA_2)$ block copolymer, which was functionalized with a DOTA ligand *via* thiol–ene chemistry. More specifically, excess maleimido-monoamide-DOTA (2.1 eq. relative to disulfide bonds) was used to convert the TEMA residues to DOTATEMA residues (Scheme 4a) to produce a PLA_{30}–$P(DMA_{30}$-*stat*-$DOTATEMA_2)$ block copolymer. The DOTA is a well-studied macrocyclic that can be used for metal conjugation. It is known to form very stable metal complexes.[73–75] The high electron density of heavy metals enables high resolution imaging of the block polymer in transmission electron microscopy (TEM) studies, as discussed below. Indium was used to form a complex with the DOTA-functionalized polymer. This was achieved by using excess $InCl_3$ (2.0 eq. relative to disulfide bonds, Scheme 4b). Successful metal complexation was confirmed by inductively-coupled plasma atomic emission spectroscopy (ICP-AES). It was calculated that there were approximately 1.16 In atoms per PLA_{30}–$P(DMA_{30}$-*stat*-$InDOTATEMA_2)$ block copolymer chain. Self-assembly of the PLA_{27}–$PGMA_{29}$ block copolymer during its synthesis in 1,2-dichloroethane was examined by TEM. Due to the insolubility of the PGMA block in this polymerization solvent the block copolymer self-assembled *in situ* to form various nanoparticles. Micelles and vesicles with diameters ranging from 50 to 500 nm were observed (see Fig. S4†).

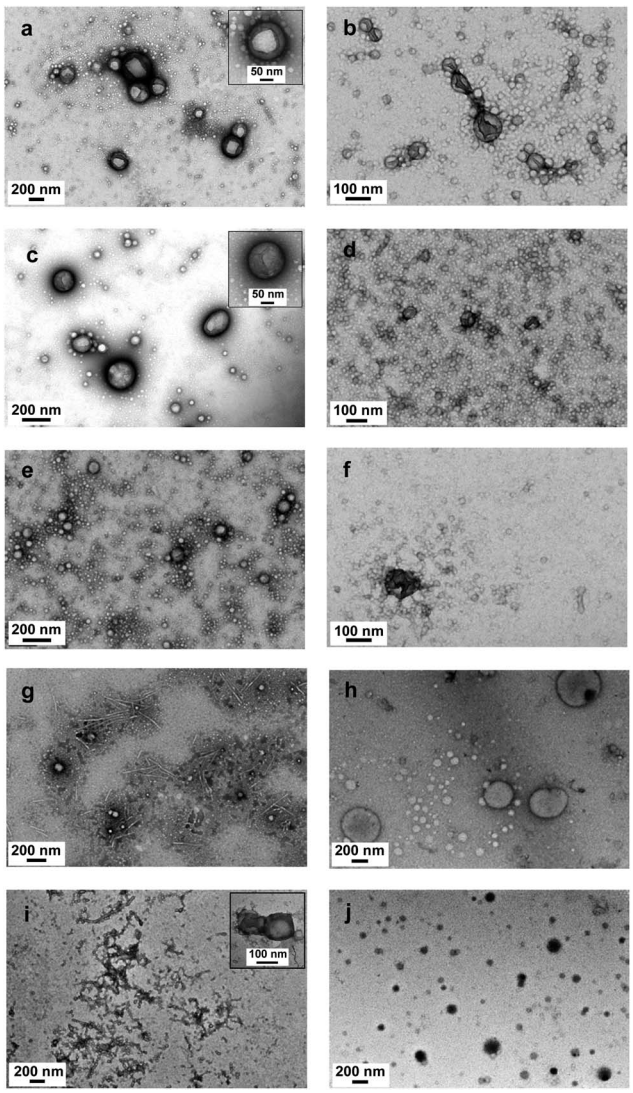


Fig. 5 TEM images obtained using a solvent switch protocol for the following diblock copolymer particles prepared by a combination of ROP and RAFT polymerizations: acetone to water solvent switch protocol was used for (a) PLA_{173}–$PDMA_{24}$; (b) PLA_{137}–$PDMA_{27}$; (c) PLA_{58}–$PDMA_{27}$; (d) PLA_{48}–$PDMA_{28}$; (e) PLA_{41}–$PDMA_{30}$; (f) PLA_{26}–$PDMA_{25}$; (g) PLA_{29}–$POEGMA_{29}$; (h) acetone/methanol (1/1 v/v) to water for PLA_{27}–$PGMA_{29}$; (i) chloroform/methanol (3/1 v/v) to water for PLA_{184}–$PMPC_{23}$; (j) acetone to water for PLA_{30}–$P(DMA_{30}$-*stat*-$InDOTATEMA_2)$. Uranyl formate was used as the positive staining agent for samples a–f, h and i. Phosphotungstic acid was the positive staining agent for sample g. No staining was used for sample j.

Self-assembly of all PLA-based amphiphilic block copolymers to form various nanostructures in water was obtained by the solvent switch method. Table 1 summarizes DLS hydrodynamic diameters observed for aqueous copolymer dispersions after removal of the organic solvent. TEM images for representative block copolymer samples are displayed in Fig. 5. Addition of water to an acetone solution of copolymer was used for the self-assembly of PLA_x–$PDMA_y$ and PLA_{29}–$POEGMA_{29}$ block copolymers. A solvent switch from acetone/methanol to water was used for the PLA_{27}–$PGMA_{29}$ and from chloroform/methanol to water for the PLA_x–$PMPC_y$ block copolymers. In the case of the PLA_x–DMA_y block copolymers, varying the PLA block DP led to different morphologies (Fig. 5a–f). Hydrodynamic diameters of these nanoparticles ranged between 113 nm and 685 nm (Table 1, entries 1 to 6). In the case of the highly asymmetric PLA_{173}–$PDMA_{24}$, vesicles make up the majority of nanoparticles (214 nm, Table 1 entry 1), but a small number of micelles were also formed (Fig. 5a). The micelle fraction increased as the DP of the PLA was reduced (from Fig. 5a–f). For the PLA_{26}–$PDMA_{25}$ block copolymer, micelles are the major population (Fig. 5f). Various nanostructures (vesicles, worm-like micelles and spherical micelles) were observed for PLA_{29}–$POEGMA_{29}$ block copolymer (Fig. 5g). The TEM image of this sample also suggested partial crystallization of the PLA block, similar to previous studies on PLA–PEG block copolymers and blends.[76–78] The same sample had a hydrodynamic diameter of 550 nm (Table 1 entry 7) and a high polydispersity. The solvent switch protocol for the PLA_{27}–$PGMA_{29}$ block copolymer resulted in a mixture of large vesicles (338 nm, Table 1 entry 9) and some micellar structures. The highly asymmetric PLA_{184}–$PMPC_{23}$ block copolymer self-assembled to produce a mixture of vesicles (1430 nm, Table 1 entry 10), worm-like micelles and spherical micelles as judged by TEM (Fig. 5i).

Following the same solvent switch protocol, the self-assembly of the In-labelled PLA_{30}–P(DMA_{30}-*stat*-InDOTATEMA$_2$) was examined. For this sample, nanostructures with a mean DLS diameter of 265 nm were observed. These morphologies could be imaged by TEM (Fig. 5j and Fig. S4a†) without requiring a staining agent. This was possible because of the high electron density of the heavy metal conjugated to the copolymer chains.

Conclusions

A combination of two "living" polymerization techniques *via* either one or two steps was achieved using a novel hydroxyl-functionalized dithiocarbonate-based ROP–RAFT dual agent. Simultaneous polymerization of an aliphatic ester and a methacrylic monomer for the one-pot synthesis of amphiphilic block copolymers has been established using a facile metal-free formulation. This was achieved by ROP of LA (catalyzed using DMAP) and RAFT polymerization of DMA or OEGMA. In contrast, a two-step approach was essential for the synthesis of PLA–GMA and PLA–PMPC block copolymers. The simultaneous ROP–RAFT polymerization protocol was also utilized for the facile synthesis of new branched amphiphilic block copolymers, which serve as precursors for thiol-functionalized linear block copolymers. For this latter synthesis, the disulfide cross-linking agent DSDMA was employed to incorporate latent thiol groups. Cleavage of the disulfide bonds was performed under reducing conditions, resulting in thiol-functionalized linear block copolymers. These biodegradable block copolymers can be conjugated with molecules such as peptides or ligands for heavy metals using facile thiol–ene chemistry, which suggests potential biomedical applications.

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