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Transformation of Thioester Initiated Star Polymers into Linear Arms *via* Native Chemical Ligation

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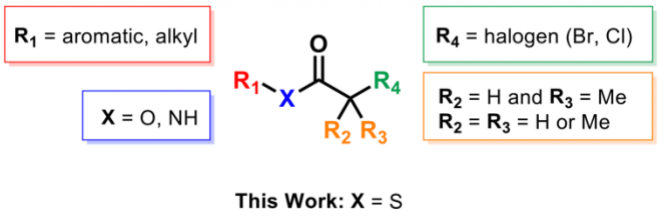
Synthesis of a new class of Cu-mediated polymerization initiators with thioester functionality is demonstrated and their polymerization kinetics *via* SET-LRP is reported. From periodic sampling, it was found that thioester or ester based initiators can be employed interchangeably, resulting in very similar polymerization rates. Furthermore, a multifunctional thioester initiator was employed, for the preparation of a well-defined four-arm star shaped polymer. It was further shown that the full dissociation of the star polymer into linear arms *via* Native chemical ligation can easily be followed *via* SEC, as a result of the change in hydrodynamic volume. Finally, the obtained linear polymers were characterized *via* MALDI-ToF MS and found to be in good agreement with the expected molecular weight distribution that confirms the successful transformation.

Thioester intermediates hold an important place in nature's synthetic toolbox, which can be found throughout various reactions, such as in native chemical ligation, formation and degradation of fatty acids, steroids, as well as in the synthesis of various coenzyme A (CoA) derivatives over acetyl-CoA and many more.[1,2] From the above, especially native chemical ligation (NCL) offers exciting possibilities in the synthesis and functionalisation of thioester containing small peptides into higher order structures, such as enzymes or proteins.[3,4] In fact, thioesters are considered to possibly be precursors to life, being obligatory intermediates in several key processes, where adenosine triphosphate (ATP) is generated or used.[5] Hence, it is not only highly desirable to incorporate thioester functionalities in biomacromolecules, but also into polymers, in order to be able to efficiently replicate biomimetic modification of polymers.

Thioester functional polymers can mainly be obtained by employing thioester containing monomers (*e.g.* thioacrylates, thiolactone), their generation during the polymerization process (*e.g.* polythioesters) or by using thioester containing chain transfer agents/initiators depending on the employed polymerisation technique.[6-10] In metal mediated polymerisations such as atom transfer radical polymerisation (ATRP) or single-electron transfer living radical polymerization (SET-LRP), mostly ester based initiators are used due to their easy synthesis, but more importantly due to their compatibility with a wide range of monomers, which is determined by efficiency of the initiator.

The employed initiators in the literature have three main structural features that effect the efficiency, namely (i) the initiator halogen, (ii) the R-groups that substitute the α-carbon and (iii) the nature of the neighbouring group (**Scheme 1**).



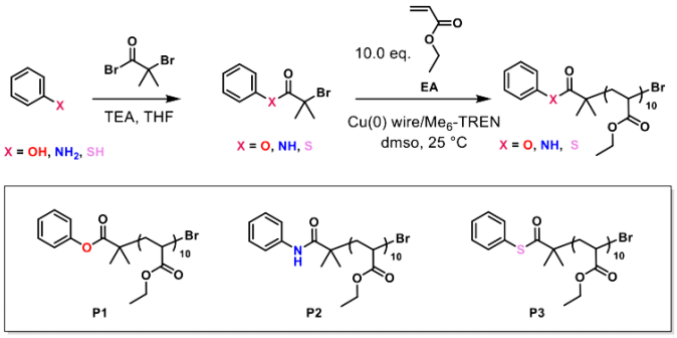
Scheme 1: General structure of a typical initiator used in ATRP or SET-LRP, and its variable components that are widely used throughout the literature.

Two of the most widely used ester initiators bearing a bromine chain end are methyl bromoproprionate and ethyl α-bromoisobutyrate (EBiB), which are mainly used to polymerise (meth)acrylates and acrylonitrile monomers.[11] Chlorine functionalised initiators on the other hand such as alkyl-2-chloropropionates are mainly utilised for the polymerisation of acrylamides and (meth)acrylates to obtain well defined polymers.[12] Similarly, phenolic-ester based initiators such as 2-bromo-2-methylpropionic acid phenyl ester or 2-chloro-2-methylpropionic acid phenyl ester find wide use in polymerisations.[13] Complimentarily, amide based initiators such as 2-chloropropionamide or 2-bromo-2-N-phenyl-propionamide can also be applied as initiators for Cu-mediated polymerisation techniques.[14,15] Last but not least, multifunctional initiators based on these structural varieties have also been widely reported in the literature and the search for functional initiator structures is still going on, as they allow the preparation of functional polymers with high chain end fidelity.[16,17]

Although there are investigations looking into activation rate constants (k_{act}) of initiators with different halogens and substituents, no evaluation of the "neighbouring group effect" (the effect of X group in **Scheme 1**) has been reported, yet.[18,19] Here, a comparison of ester, thioester and an amide initiator is made for the first time to investigate their polymerisation behaviour. For this, SET-LRP conditions were used to polymerise ethyl acrylate (EA) as a model monomer. Next, a thioester based

tetrafunctional initiator was used to obtain a star shaped polymer, which was subsequently dissociated into a linear polymer *via* native chemical ligation (NCL) over the thioester moieties using L-cysteine methyl ester hydrochloride (Cys-ME).

Initially, due to the commercial availability, a series of phenylic initiators were prepared, which allowed the direct comparison of their polymerisation kinetics under identical reaction conditions. For this purpose, phenol, thiophenol and aniline were transformed into initiators using α-Bromoisobutyryl bromide (**Scheme 2**). The successful synthesis thereof was confirmed *via* NMR spectroscopy (*i.e.* shift of the corresponding phenyl -CH signal) and mass spectrometry (see SI Experimental section for detailed description for their synthesis and full product characterisation). The obtained initiators were used to polymerise ethyl acrylate (DP=10) at 25 °C using a pre-activated Cu(0)-wire and [M]:[I]:[$CuBr_2$]:[Me_6TREN] = 10:1:0.1:0.19.



Scheme 2: General synthesis route for the ester, amide, thioester initiators and their use in the polymerisation of ethyl acrylate (**P1-P3**).

The polymerisation kinetics were followed for 150 minutes and aliquots were withdrawn periodically to determine the monomer conversion and molar mass distribution. For the first 40 minutes of the polymerisation, the conversion for all polymers were calculated to be below 10%, indicative of the presence of an induction period (**Figure 1**). Several potential reasons of the induction period in Cu mediated polymerisation have been reported in the literature, mainly around the presence of a

passivating Cu_2O sheet on the wire surface and the accumulation of dissolved copper species, that act as a deactivator (Cu^{II}).[20,21] However, it should be noted that copper wire employed in this study is pre-activated with HCl and should not be contributing towards the presence of an induction period. While the conversion for the amide based initiator (PhNHBIB) remains low (14%), quantitative conversion was obtained for the ester- and thioester based initiators after 150 minutes. This low conversion for PhNHBIB can be attributed to the general low initiator efficiency of amide based initiators (*i.e.* stronger bond between the carbon-halogen). A similar observation can be made when using acrylamides in a copper-based polymerization system, where a complexation between the copper and amide group might occur. The apparent kinetic rate constants k_p^{app} were calculated after the induction period and were indicative of almost identical monomer consumption rates (**P1:** PhBIB = 0.032 min^{-1}, **P2:** PhNHBIB = 0.002 min^{-1}, while for **P3:** PhSBIB = 0.031 min^{-1}).

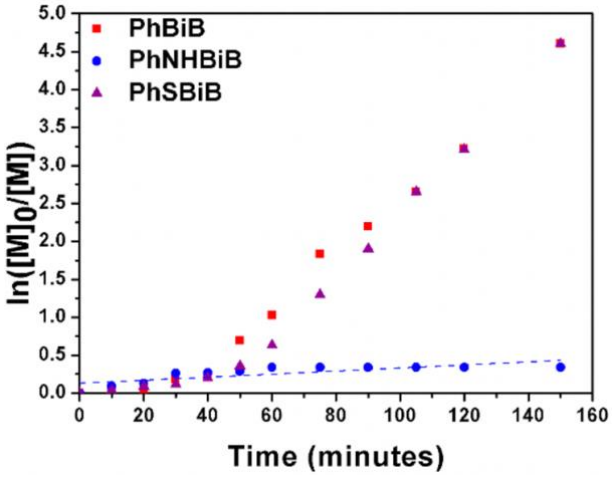
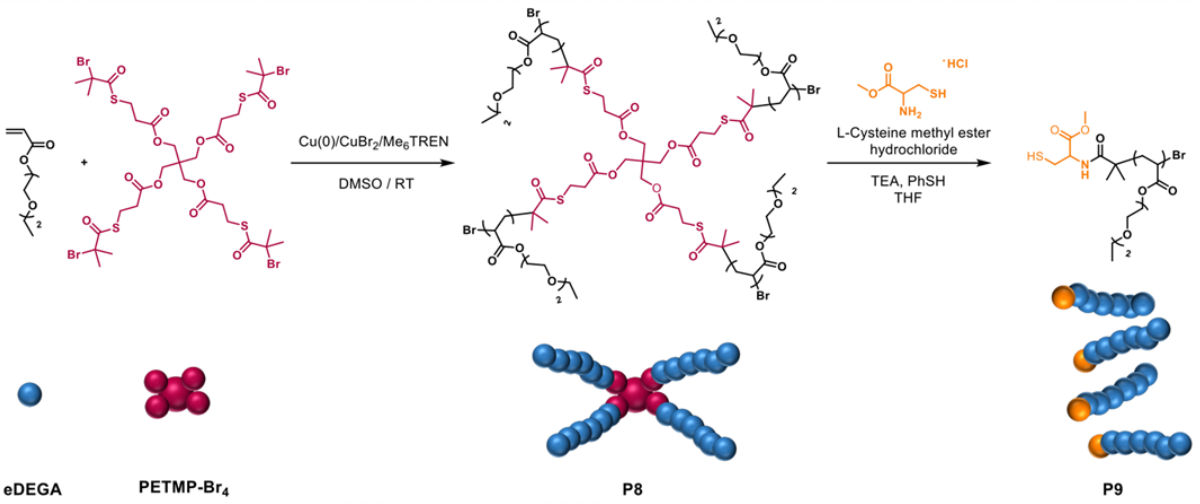


Figure 1: First order kinetic plots for all three different initiators obtained from periodic sampling during polymerisation with [EA]:[I]:[$CuBr_2$]:[Me_6TREN] = 10:1:0.1:0.19.

In order to exclude the effect of the aromatic ring, a comparison of EBiB (**P4**) with its thioester counterpart *S*-EBiB (**P5**) was carried out, yielding almost identical results for the first linear region of the kinetic plot (**Figure S10**). However, after 90 minutes of

polymerisation, monomer conversions in both reactions started to level off, resulting in quantitative monomer conversions in 120 minutes for **P4** and 240 minutes for **P5**. Thus, the above obtained results for the different initiators indicate that, where an amide based initiator is not sufficient to polymerise acrylates, an ester and a thioester initiator can be used interchangeably. A prolonged reaction time for the *S*-EBiB initiated system should be noted.

Parallel to the investigation of linear thioester initiators mentioned earlier, the first example of the synthesis of a thioester based star initiator is also demonstrated here by conversion of a tetrathiol functional molecule to a tetrathioester initiator PETMP-Br_4. The functionalisation of the thiol groups and the successful synthesis of the initiator was confirmed *via* NMR spectroscopy (**Figure S7-S8**). Initially, di(ethylene glycol) ethyl ether acrylate (eDEGA) was polymerised using the conditions above, with [M]:[I]=100 for **P6** and [M]:[I]=200 for **P7** (**Scheme 3**). The obtained gel permeation chromatography (GPC) traces show a rapid increase in molecular weight, especially after 45 minutes into the polymerisation, after which coupling reactions start to occur, as evidenced by the appearance of a second signal at higher molecular weight (**Figure S11**). Similarly, the semi logarithmic kinetic plot of **P6** and **P7** displays a somewhat linear increase in the initial 45 minutes of the polymerization (**Figure S12**). After 60 minutes, an accelerated second linear region is observed, which after 90 minutes slopes down until the polymerization stops. The decrease in rate could be attributed to the increase in $CuBr_2$ species, due to the irreversible termination that occurs from coupling reactions. Nevertheless, 97% and 93% monomer conversion were reached for **P6** and **P7,** respectively, in three hours (see **ESI section 3.2.2**). This clearly shows that tetrafunctional thioester based initiators, allow the polymerisation of star shaped acrylates to high molecular weight *via* SET-LRP.



Scheme 3: Schematic representation of the polymerisation of eDEGA using a thioester star initiator (PETMP-Br_4) yielding **P8** and its controlled dissociation into linear arms of **P9** *via* NCL with L-Cysteine methyl ester hydrochloride (Cys-ME) catalysed by TEA and thiophenol.

In nature, thioesters are functional precursors for chemoselective amidation reactions with amines, especially *via* native chemical ligation. For example, this transfer reaction with cysteine is well explored and understood.[22,23] Therefore, it was hypothesised that a thioester containing polymer could undergo amidation with amine groups or be conjugated to a peptide. To be able to facilitate the undocking of the arms from the core, possible model reactions that involve modification of thioesters were explored. Carrying out such ligation chemistry, could allow direct conjugation of a polymer to peptides over cysteine residues, which is otherwise known to be challenging.[24]

In addition, cysteine could be used to force a transition of a thioester initiated star polymer to a linear polymer. For this purpose, the star polymerisation of $(eDEGA)_{200}$ was intentionally stopped in another experiment after one hour, to result in a well-defined polymer without any presence of side reactions (**P8**, $M_{n,GPC}$ = 9300 g/mol, PDI = 1.14, **Figure S13**). The obtained polymer was purified *via* dialysis to ensure removal of any residual monomer in solution (**Figure S14**).

Next, **P8** was dissolved in THF and test reactions were conducted using benzylamine for a simple amidation reaction, in the absence and excess of triethyl amine (TEA) and thiophenol (as base and catalyst respectively). Periodic GPC samples up to 24 hours have neither revealed a change in the shape of the **P8** trace, nor were any signals for lower molecular weight polymers, associated to the separation of the linear arms, detected. Thus, it was concluded that the use of a base and catalyst in this time span with an amine under these conditions alone is not sufficient to carry out an amidation reaction on the thioester moieties within the core. Similarly, no reaction was observed, when Cys-ME was added to the polymer solution. However, complete dissociation of the star polymer into its arms was observed, once Cys-ME was introduced in the presence of TEA and thiophenol, which has earlier been shown to catalyse the amidation reaction (**Scheme 3**).[25] The progress of this reaction could easily be followed *via* GPC, by the gradual decrease of the **P8** trace and increase of the newly formed polymeric species (**P9**) at higher retention times (**Figure 2**).

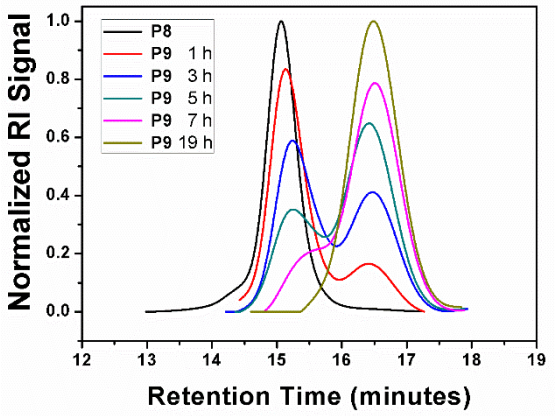


Figure 3: Overlay of the GPC traces obtained during the dissociation of the star polymer **P8**, displaying a shift towards higher retention times.

The black trace in **Figure 3** represents the initial star polymer **P8**. Already within the first hour, a new signal starts appearing with a retention time of around 16.5 minutes assigned to **P9** (red trace). This new trace appears to have a higher signal intensity compared to **P8** after 5 hours, showing a star dissociation of more than 50% (visual estimate from peak heights).

After 19 hours, the signal for **P8** has completely disappeared, indicative of completion of the NCL reaction. **P9** was obtained and analysed to be a well-defined polymer ($M_{n,GPC}$ = 2800 g/mol, PDI = 1.10). In order to be able to match the newly appearing trace to that of the product, an aliquot from the reaction mixture was submitted to MALDI-ToF MS analysis. The obtained spectrum is displayed below in **Figure 3** (See **Figure S15** for full spectrum).

The mass spectrum in **Figure 3** displays periodically reoccurring peaks with a separation of 188.2 *m/z*, which matches with the monomer eDEGA. When the masses were analysed, two main distributions were observed for the polymer, with the ω-terminus bearing –Br, as the expected mass distribution for **P9** (denoted $\mathbf{A_{DP}\text{-}Br}$), but also with –H, indicating some loss of chain end fidelity during the initial polymerisation (denoted $\mathbf{A_{DP}\text{-}H}$). The observed masses further confirm the presence of the Cys-ME end group on the α-terminus (all masses labelled **A** in **Figure 3**). Finally, peaks labelled as **B** were identified as species with the thiol to be oxidised to a sulfinic acid group, corresponding to an observed mass increase of 32 *m/z*. Although multiple peaks were detected in the mass spectrum, it was concluded that all peaks corresponded to the same polymer distribution, hence proving the successful undocking of the linear arms from star polymer **P8** *via* NCL.

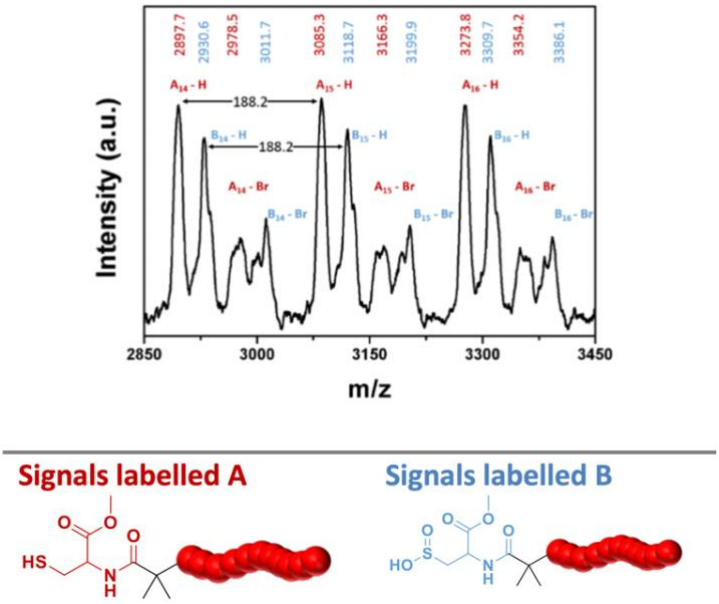


Figure 3: MALDI-ToF MS spectrum obtained from the reaction of **P8** after 19 hours, displaying the product **P9**. Two main distributions are evident, which are matched to be linear chains with the α-terminus bearing **A)** free thiol group and **B)** sulfinic acid group. (See **Table S4** for full list of peaks and assignment.)

In this report, a new class of thioester based initiators that can be used in Cu-mediated polymerisations are reported. It is shown that thioester based initiators show identical polymerisation behaviour as ester based initiators, when used for the polymerisation of acrylates. An interference between the thioester groups and the catalyst system was not observed. Thus thioester initiators can be employed alike ester initiators. We have further adapted this concept to multifunctional thioester initiators, in order to obtain high molecular weight star polymers *via* the core-first approach. Upon the introduction of L-Cysteine methyl ester, the star polymer underwent a transformation into its linear arms *via* native chemical ligation. We believe that this example can be adapted for conjugation of polymers in an efficient way to various amino acids or peptides that contain cysteine residues.

Supporting Information
Supporting Information includes initiator synthesis, all polymerization procedures and further characterization, and is available from the Wiley Online Library or from the author.

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Conflict of Interest
The authors declare no conflict of interest.

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Table of contents

Comparing different initiator types for Cu-mediated polymerization, has shown that thioester based initiators can be used as an excellent alternative that allow further conjugation *via* native chemical ligation as exemplified on a star polymer using L-cysteine methyl ester hydrochloride.

Keyword

Copper mediated polymerization, star polymers, thioester initiators, native chemical ligation, NCL

Authors

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Title

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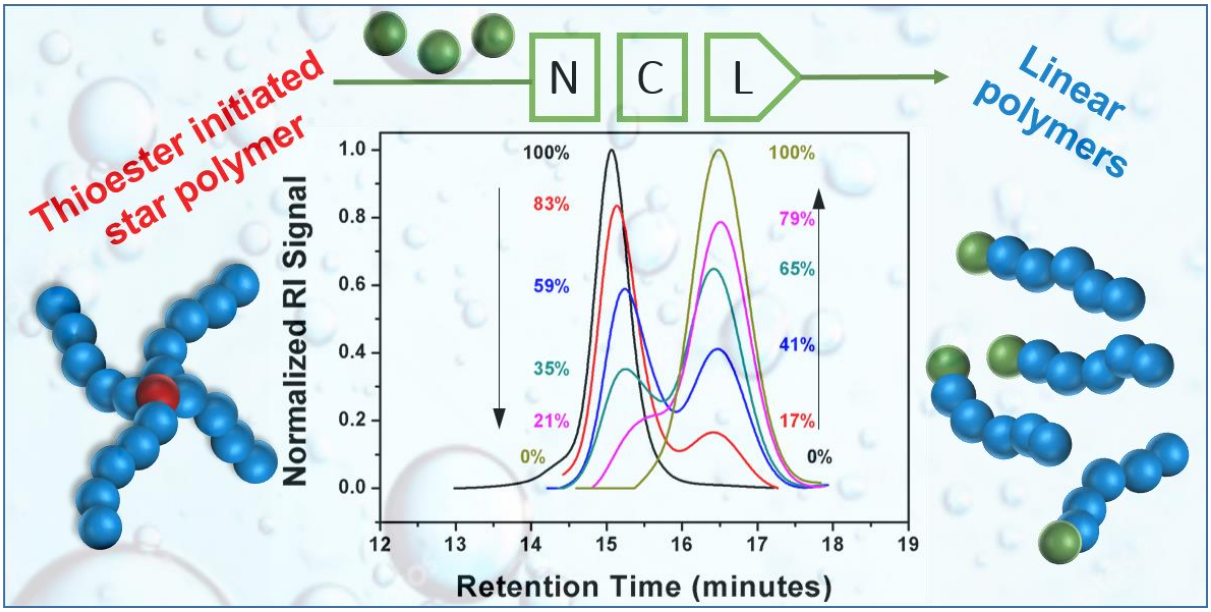

Supporting Information

Transformation of Thioester Initiated Star Polymers into Linear Arms *via* Native Chemical Ligation

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1. Materials

All reactions involving air- and/or moisture-sensitive substances were carried out under an inert atmosphere (argon or nitrogen) using standard Schlenk techniques. Solvents were dried and degassed using standard laboratory techniques.

All monomers, solvents and chemicals were purchased from Sigma-Aldrich (UK) at highest purity available. Commercially available monomers were destabilized by passing through a short column of basic aluminium oxide prior to polymerization.

2. Instruments

2.1 Gel permeation chromatography (GPC)

Molecular weight averages and polymer dispersity of obtained polymers were determined by GPC in THF. Measurements were performed on an Agilent 390-LC system equipped with a PL-AS RT autosampler, 2PLgel 5 μm mixed-C columns (300×7.5 mm), a PLgel 5 mm guard column (50×7.5 mm), and a differential refractive index (DRI). The system was eluted with THF containing 2% trimethylamine (TEA) at a flow rate of 1 mL min-1 and the DRI was calibrated with linear narrow poly(methyl methacrylate) standards in range of 1010 to 2136000 g/mol. All samples were passed through neutral aluminium oxide to remove any catalyst residues and filtered using 0.2 μm PTFE filters before analysis.

2.2 Nuclear magnetic resonance (NMR)

^{1}H NMR spectra were recorded on a Bruker AV-400 or Bruker Avance 600 spectrometer at 303K. The resonance signal at 7.26 ppm (^{1}H) was used as residual $CDCl_3$ or for $(CD_3)_2CO$ at 2.05 ppm peak for the chemical shift (δ). For ^{13}C NMR spectra were referenced relative to the solvent signal (77.16 ppm).

2.3 Mass spectrometry (MS)

High-resolution mass spectra (HRMS) were recorded on a Waters SYNAPT G2-Si High Definition Mass Spectrometry system using a solvent gradient (0->100% Acetonitrile in Water + 0.1% Formic acid) in positive electrospray ionisation (ESI+) mode equipped with an Acquity UPLC BEH C18 column (2.1 x 50 mm; 130Å). The instrument was tuned using a Leucin Enkephalin mix to optimum resolution and signal intensity and was calibrated using a Waters Major Mix IMS/ToF in a range of m/z 50-1200.

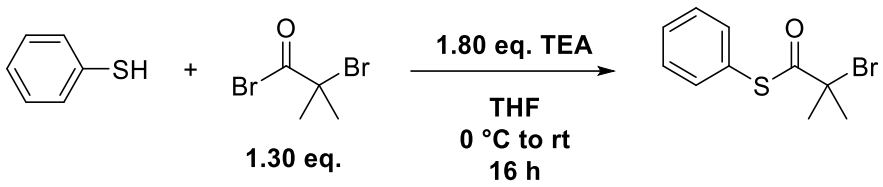
2.4 Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-ToF-MS)

MALDI-TOF MS was performed using a Bruker Daltonics Autoflex MALDI-ToF mass spectrometer, equipped with a nitrogen laser at 337 nm with positive ion ToF detection. Polymer samples were measured as follows; solutions in THF of trans-2-[3-(4-tert-Butylphenyl)-2-methyl-2-propenylidene]malononitrile (DCTB, ≥98%) as matrix (30 mg/ml), silver trifluoroacetate (AgTFA) as cationisation agent (10 mg/ml) and sample (10 mg/ml) were mixed together in a 9:1:1 volume ratio for a total volume of 75 μL. 2 μL of the mixture was applied to the target plate. Spectra were recorded in reflectron mode and the mass spectrometer was calibrated with a peptide mixture up to 6000 Da.

3 Experimental Procedures

3.1 Synthesis and characterisation of the initiators

3.1.1 Synthesis of S-phenyl 2-bromo-2-methylpropanetioate (PhSBiB)



In a 500 mL 2-neckround bottom flask, thiophenol (10 mL, 97.39 mmol) and TEA (24.34 mL, 174.62 mmol) were stirred in dry THF (100 mL) and cooled down to 0°C in an ice-bath. A mixture of BIBB (17.98 mL, 145.98 mmol) and THF (50 mL) were added dropwise over a period of 1 hour under argon. The mixture was then allowed to warm up to ambient temperature and stirred for 15 h. The precipitated salt was removed *via* filtration and washed with 60 mL of THF. The filtrate was collected an concentrated in *vacuo*, dissolved in DCM, washed with 10% HCl (3 x 100 mL), 5% NaOH (3 x 100 mL), brine (3 x 100 mL) and passed over a column of basic aluminium oxide to remove any impurities to yield a viscous pale yellow oil. (**Yield**=14.88 g, 59%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.86 (s, H_d, 6H), 7.06-7.35 ppm (H_a, H_b, H_c, 5H).
^{13}C NMR ($CDCl_3$, 400 MHz) δ: 30.79, 64.96, 127.36, 130.12, 135.22, 197.04.
HRMS (m/z): $C_{10}H_{11}BrOS$, calc.: 257.9714, found: 258.9671 $[MH]^+$

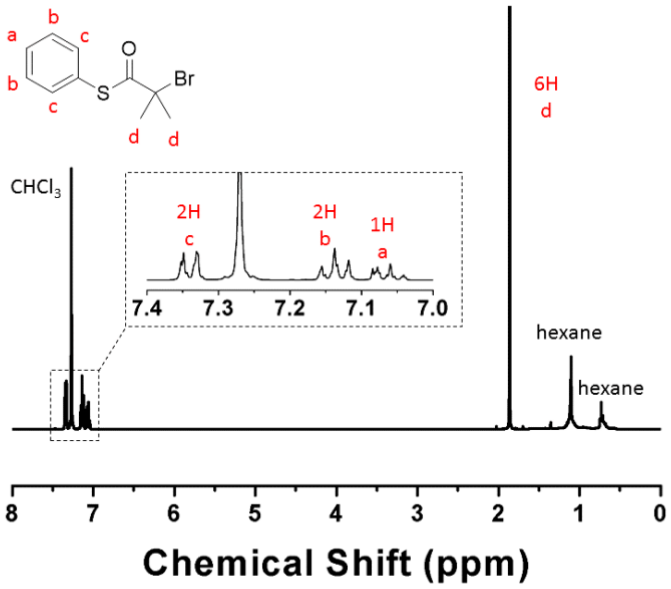


Figure S1: [1]H NMR spectrum obtained for PhSBiB.

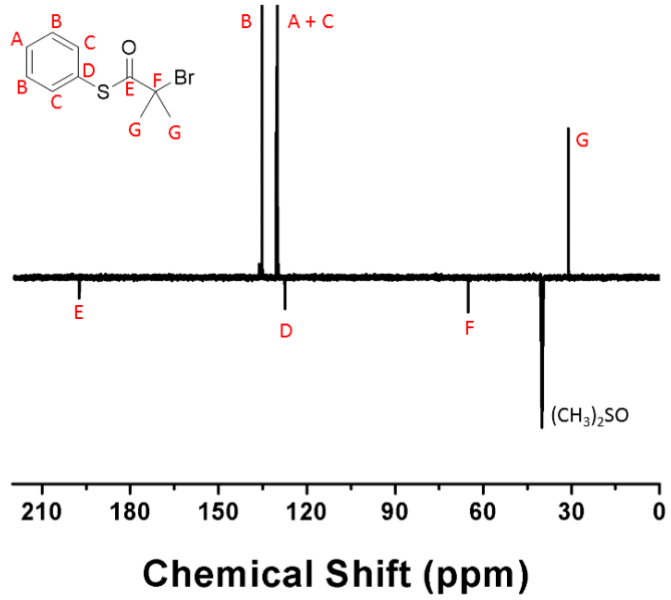
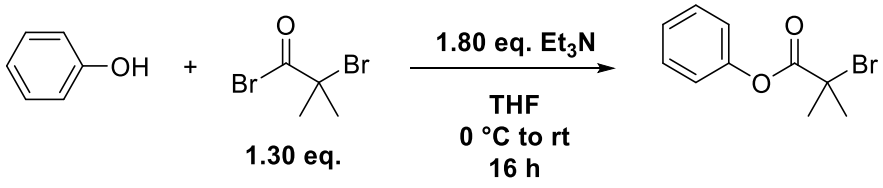


Figure S2: [13]C NMR spectrum obtained for PhSBiB.

3.1.2 Synthesis of Phenyl 2-bromo-2-methylpropanoate (PhBiB)



In a 500 mL 2-neckround bottom flask, phenol 5.00 g, 53.13 mmol) and NEt_3 (13.00 mL, 92.68 mmol) were stirred in dry THF (100 mL) and cooled down to 0°C in an ice-bath. A mixture of BIBB (8.50 mL, 69.06 mmol) and THF (50 mL) were added dropwise over a period of 1 hour under argon. The mixture was then allowed to warm up to ambient temperature and stirred for 15 h. The precipitated salt was removed *via* filtration and washed with 60 mL of THF. The filtrate was collected an concentrated in *vacuo*, dissolved in DCM, washed with 10% HCl (3 x 100 mL), 5% NaOH (3 x 100 mL), brine (3 x 100 mL) and passed over a column of basic aluminium oxide to remove any impurities to yield a pale brown solid. (**Yield**=10.61 g, 82%).

^{1}H NMR ($(CH_3)_2SO$, 400 MHz) δ = 2.05 (s, H_d, 6H), 7.18 – 7.47 (m, H_a, H_b, H_c, 5H)
^{13}C NMR ($(CH_3)_2SO$, 400 MHz) δ: 30.22, 57.28, 121.28, 126.44, 129.83, 150.57, 169.68 ppm.
HRMS (m/z): $C_{10}H_{11}BrO_2$, calc.: 241.9942, found: 242.0022 $[MH]^+$

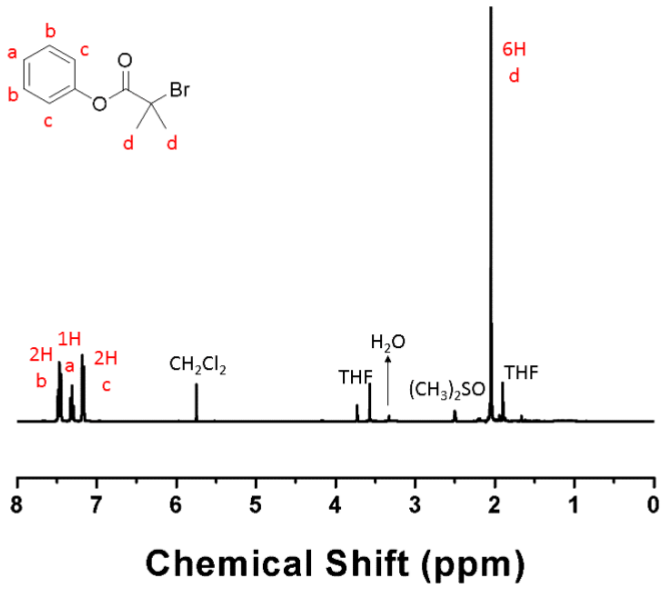


Figure S3: ^{1}H NMR spectrum obtained for PhBiB.

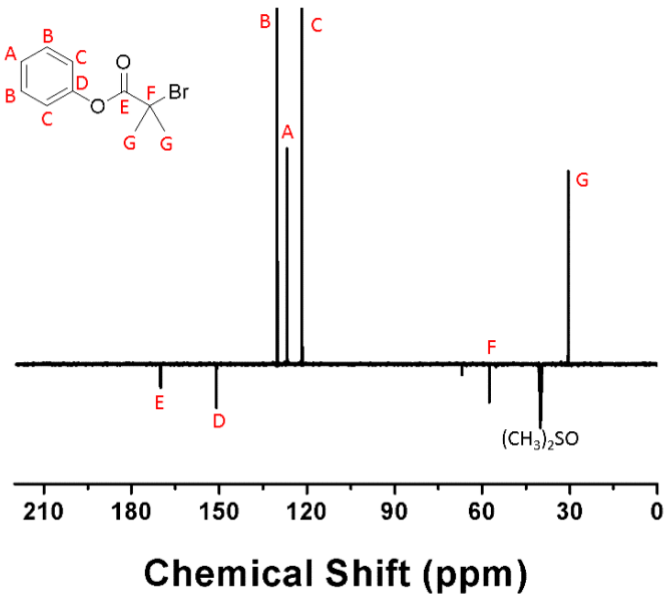
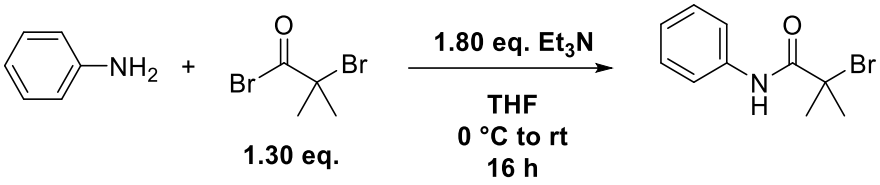


Figure S4: ^{13}C NMR spectrum obtained for PhBiB.

3.1.3 Synthesis of Phenyl 2-bromo-2-methylpropanoate (PhNHBiB)



In a 500 mL 2-neckround bottom flask, aniline 10.2 g, 109 mmol) and NEt_3 (24.3 mL, 174 mmol) were stirred in dry THF (150 mL) and cooled down to 0°C in an ice-bath. A mixture of BIBB (30.4 mL, 247 mmol) and THF (50 mL) were added dropwise over a period of 1 hour under argon. The mixture was then allowed to warm up to ambient temperature and stirred for 15 h. The precipitated salt was removed *via* filtration and washed with 200 mL of THF. The filtrate was collected an concentrated in *vacuo*, dissolved in DCM, washed with 10% HCl (3 x 150 mL), 5% NaOH (3 x 150 mL), brine (3 x 150 mL) and passed over a column of basic aluminium oxide to remove any impurities to yield a pale brown solid. (**Yield** = 23.9 g, 90%).

^{1}H NMR ($(CH_3)_2SO$, 400 MHz) δ = 2.00 (s, H_e, 6H), 7.11 – 7.64 (m, H_a, H_b, H_c, 5H), 9.77 ppm (s, H_d, 1H).
^{13}C NMR ($(CH_3)_2SO$, 400 MHz) δ: 30.79, 60.56, 120.31, 123.68, 128.54, 138.54, 169.35 ppm.
HRMS (m/z): $C_{10}H_{12}BrNO$, calc.: 241.0102, found: 241.0189 $[MH]^+$

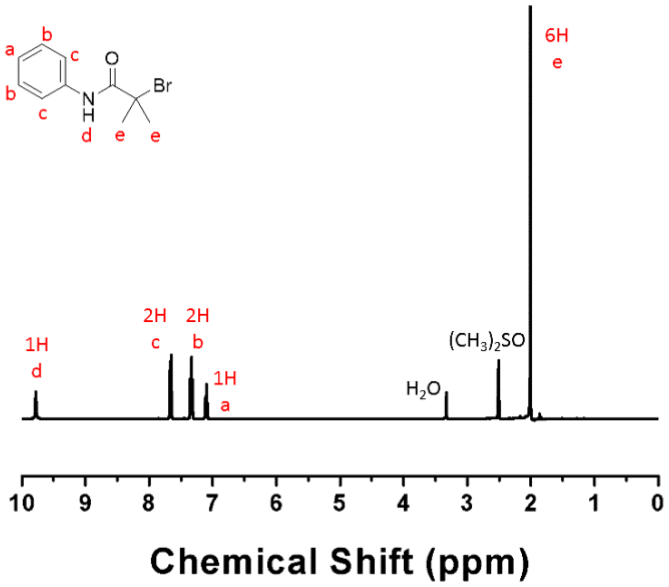


Figure S5: ^{1}H NMR spectrum obtained for PhNHBiB.

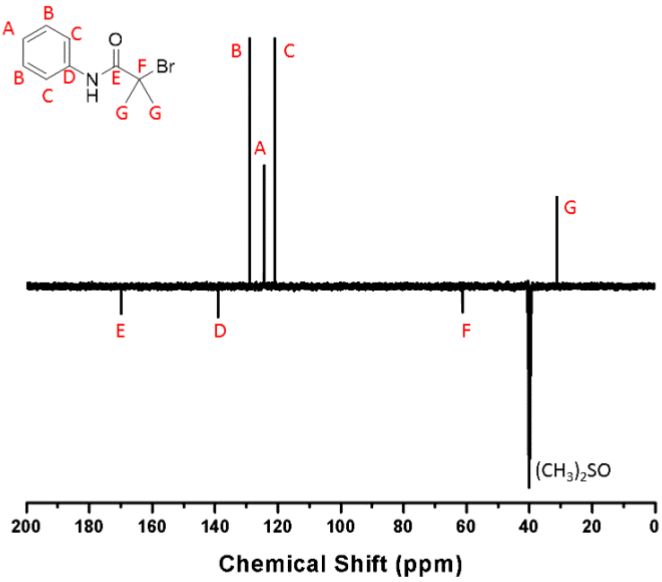
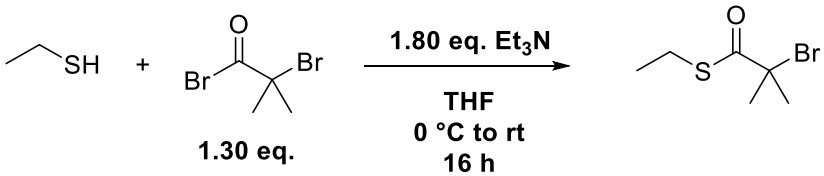


Figure S6: ^{13}C NMR spectrum obtained for PhNHBiB.

3.1.4 Synthesis of S-ethyl 2-bromo-2-methylpropanethioate (*S*-EBiB)



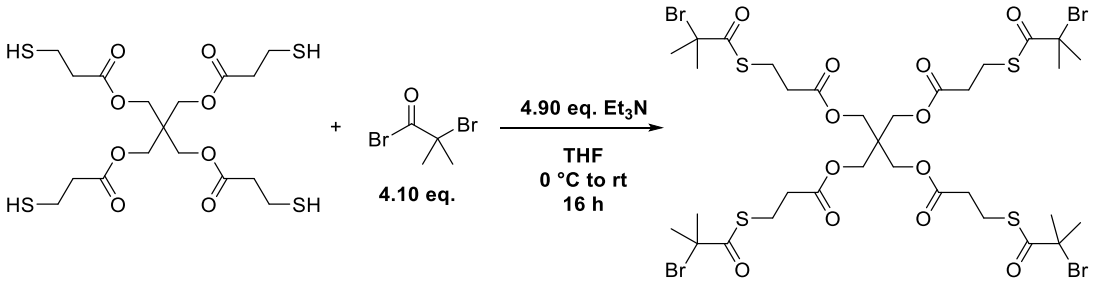
In a 500 mL 2-neckround bottom flask, ethanethiol (3.60 mL, 48.65 mmol) and NEt_3 (12.17 mL, 87.31 mmol) were stirred in dry THF (100 mL) and cooled down to 0°C in an

ice-bath. A mixture of BIBB (8.99 mL, 72.99 mmol) and THF (50 mL) were added dropwise over a period of 1 hour under argon. The mixture was then allowed to warm up to ambient temperature and stirred overnight. The precipitated salt was removed via filtration and washed with 60 mL of THF. The filtrate was collected an concentrated in vacuo, dissolved in DCM, washed with 10% HCl (3 x 30 mL), 5% NaOH (3 x 30 mL), brine (3 x 30 mL) and passed over a column of basic aluminium oxide to remove any impurities to yield a viscous pale yellow oil. (**Yield** = 4.90 g, 48%)

^{1}H NMR (CDCl3, 400 MHz) δ: 2.89 (q, 3H), 194 (s, 6H), 1.27 ppm (t, 2H).

^{13}C NMR (CDCl3, 400 MHz) δ: 200.02, 64.25, 31.23, 24.59, 14.24 ppm.

3.1.5 Synthesis of Pentaerythritol tetrakis 3-mercaptoprionate-2-bromoisobutyrate (PETMP-Br$_4$)



In a 500 mL 2-neckround bottom flask, Pentaerythritol tetrakis(3-mercaptopropionate) (7.00 mL, 17.74 mmol) and Et_3N (12.17 mL, 87.85 mmol) were stirred in dry THF (100 mL) and cooled down to 0°C in an ice-bath. A mixture of BIBB (8.99 mL, 72.93 mmol) and THF (50 mL) were added dropwise over a period of 1 hour under argon. The mixture was then allowed to warm up to ambient temperature and stirredfor 15 h. The precipitated salt was removed *via* filtration and washed with 60 mL of THF. The filtrate was collected an concentrated in *vacuo*, dissolved in DCM, washed with 10% HCl (3 x 100 mL), 5% NaOH (3 x 100 mL), brine (3 x 100 mL) and passed over a column of basic aluminium oxide to remove any impurities to yield a viscous pale yellow oil. (**Yield** = 9.60 g, 50%).

$M_{n,GPC}$ = 1020 gmol^{-1} , (PDi = 1.03).
^{1}H NMR ($CDCl_3$, 400 MHz) δ: 4.17 (s, 8H), 3.14 (t, 8H), 2.68 (t, 8H), 1.96 (s, 24H) ppm.
^{13}C NMR ($CDCl_3$, 400 MHz) δ: 24.67, 30.79, 33.46, 41.77, 77.04-76.70, 170.97, 199.43 ppm.
HRMS (m/z): $C_{33}H_{48}Br_4O_{12}S_4$, calc.: 1079.8762, found: 1080.8849 $[MH]^+$

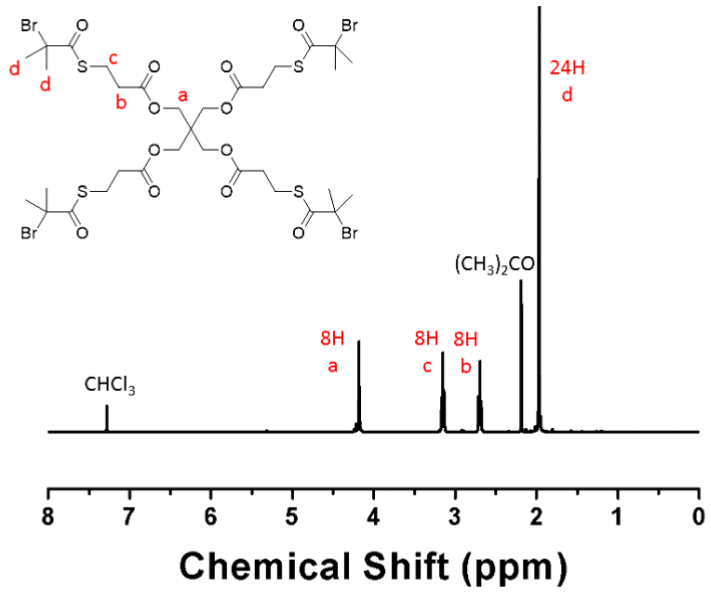


Figure S7: ^{1}H NMR spectrum obtained for PETMP-Br_4.

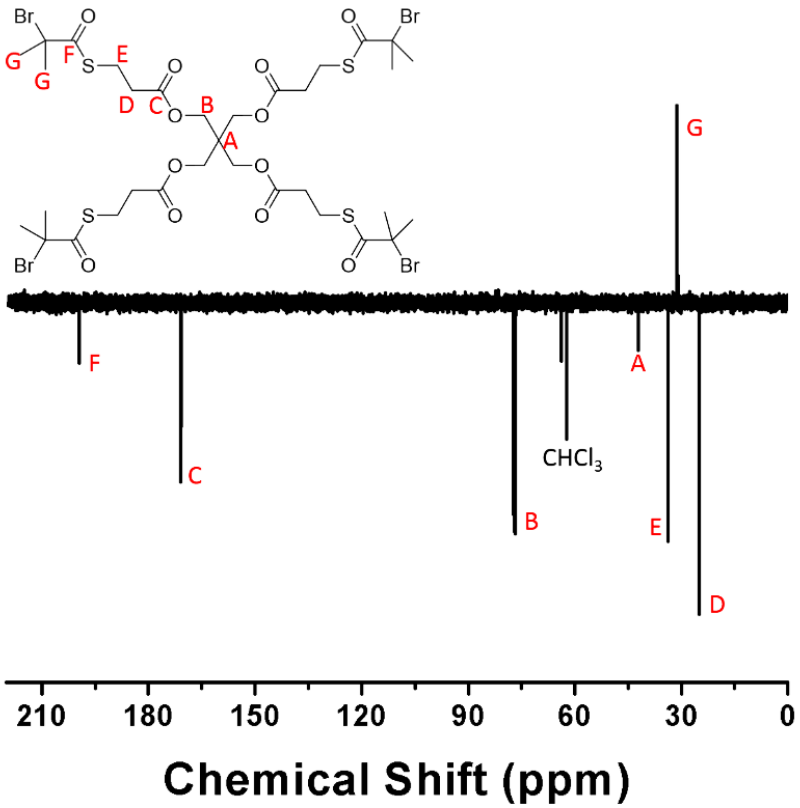


Figure S8: ^{13}C NMR spectrum obtained for PETMP-Br_4.

3.2 Polymerization Procedures

3.2.1 General procedure for the homopolymerisation of EA_{10} (P1-P5)

For a typical polymerisation, $CuBr_2$ (0.1 eq.), DMSO (1:2 *v*/*v*), Me_6TREN (0.19 eq.), EA (10 eq.), mesitylene as an internal standard (2.5%, *v*/*v*) and initiator (1.0 eq.) were added to a Schlenk tube containing a stirrer bar. The Schlenk tube was subsequently sealed with a rubber septum, lowered into an oil bath set to 25 °C and degassed with argon for 30 minutes. At the same time, the copper wire was preactivated in 10 mL HCl (conc. 37%) for 20 minutes, then washed with deionised water, acetone and dried under argon. The activated copper wire was then immediately transferred to the Schlenk tube containing the polymerisation mixture to start the reaction (the addition of the copper wire defines t = 0).

Table S1: Overview of the amounts used for the polymerisation of **P1-P3**.

Polymer	EA (g)	Initiator	Initiator (mg)	Me_6TREN (µL)	$CuBr_2$ (mg)	DMSO (mL)
P1	1.00	PhBiB	242	50.7	22.3	2.00
P2	1.00	PhNHBiB	241	50.7	22.3	2.00
P3	1.00	Ph*S*-BiB	259	50.7	22.3	2.00
P4	1.00	EBiB	195	50.7	22.3	2.00
P5	1.00	*S*-EBiB	211	50.7	22.3	2.00

Polymerisation conditions: [EA]:[I]:[$CuBr_2$]:[Me_6TREN] = 10:1:0.1:0.19 with 4 cm Cu(0)wire at 25 °C.

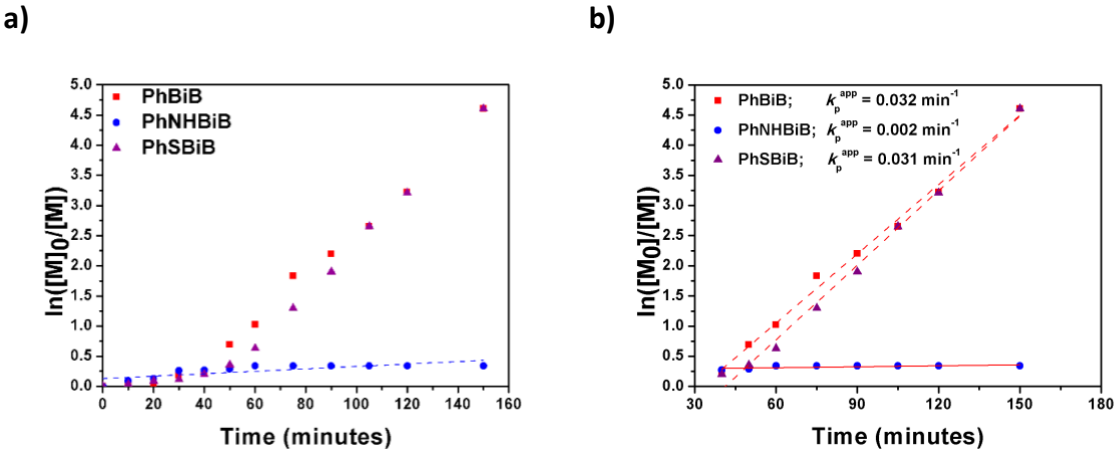


Figure S9: a) First order kinetic plots for all three different initiators obtained from periodic sampling during polymerisation with [EA]:[I]:[$CuBr_2$]:[Me_6TREN] = 10:1:0.1:0.19. **b)** Calculated apparent kinetic rate constants (k_p^{app}) after the induction period (40 minutes onwards).

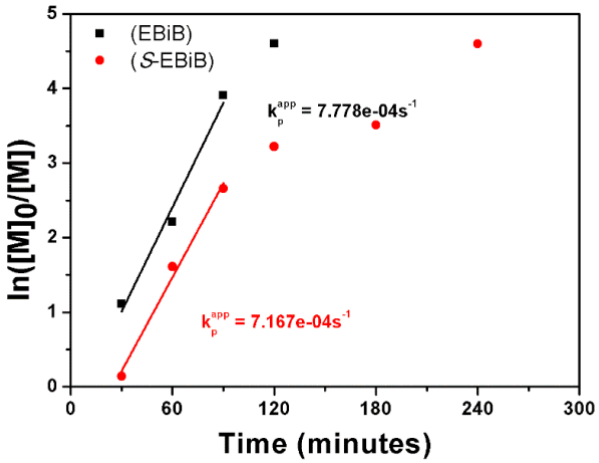


Figure S10: Comparison of EBiB (**P4**) and *S*-EBiB (**P5**) used for polymerisations of EA. [EA]:[I]:[Me_6TREN]:[$CuBr_2$] = 10:1:0.19:0.1 in DMSO.

3.2.2 General procedure for the star polymerisation of eDEGA (P6-P7)

For a typical polymerisation, $CuBr_2$ (0.1 eq.), DMSO (1:2 *v*/*v*), Me_6TREN (0.19 eq.), eDEGA (100 or 200 eq.), mesitylene (2.5%, *v*/*v*) and PETMP-Br_4 star initiator (1.0 eq.) were added to a Schlenk tube containing a stirrer bar. The Schlenk tube was subsequently sealed with a rubber septum, lowered into an oil bath set to 25 °C and degassed with argon for 30 minutes. At the same time, the copper wire was preactivated in 10 mL HCl (conc. 37%) for 20 minutes, then washed with deionised water, acetone and dried under argon. The activated copper wire was then immediately transferred to the Schlenk tube containing the polymerisation mixture to start the reaction (the addition of the copper wire defines t = 0).

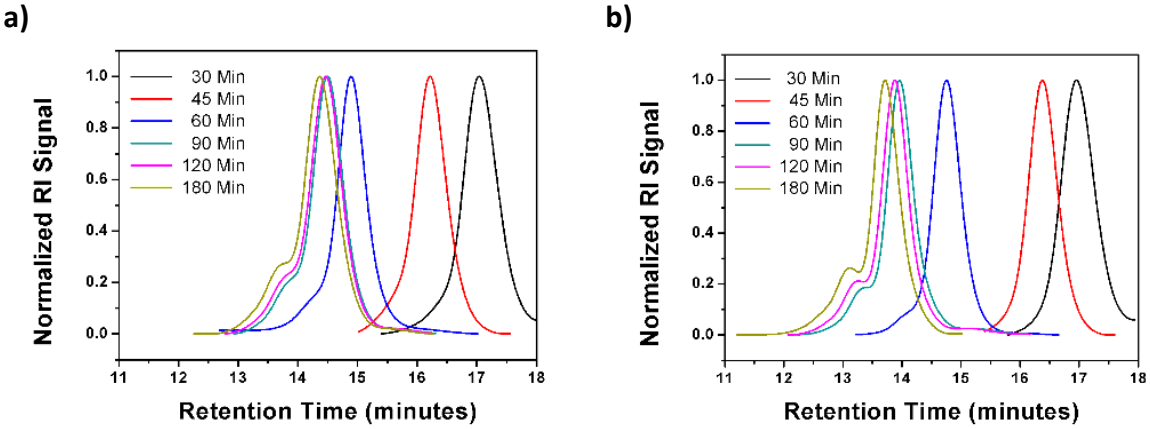


Figure S11: Overlay of the GPC traces obtained from kinetic sampling for **a) P6** and **b) P7.**

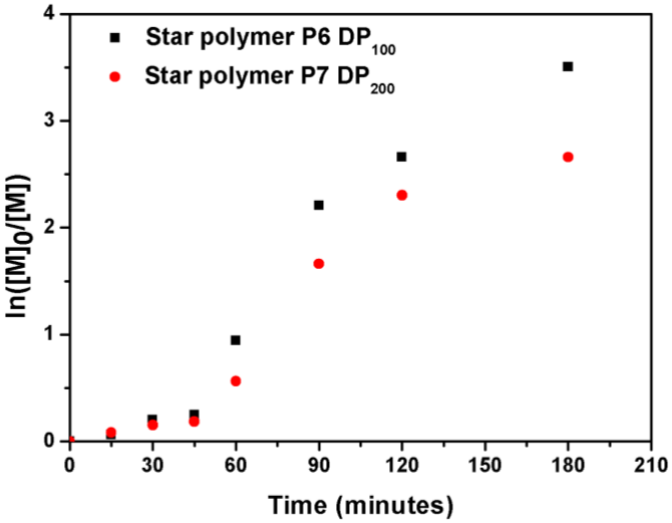


Figure S12: Semi logarithmic kinetic plot obtained from periodic sampling during polymerisation of eDEGA using star initiator PETMP-Br$_4$ with [M]:[I] = 100 for **P6,** and [M]:[I] = 200 for **P7**.

Table S2: Overview of the GPC results obtained during the polymerisation of **P6.**

Time (min)	$M_{n,theo}$ (g/mol)	$M_{n,GPC}$[b] (g/mol)	PDI[b]	Conv.[a] (%)
15	2210	N/A	N/A	6
30	4470	1740	1.14	18
45	5220	3550	1.24	22
60	12560	11040	1.37	61
90	17840	15660	1.33	89
120	18590	16310	1.21	93
180	19340	18100	1.10	97

Polymerisation condition: [eDEGA]:[I]:[$CuBr_2$]:[Me_6TREN] = 100:1:0.1:0.19. [a]Conversion measured by ^{1}H NMR spectroscopy. [b] THF eluent, linear PMMA standard.

Table S3: Overview of the GPC results obtained during the polymerisation of **P7.**

Time (min)	$M_{n,theo}$ (g/mol)	$M_{n,GPC}$[b] (g/mol)	PDI[b]	Conv.[a] (%)
15	4090	N/A	N/A	8
30	6350	1860	1.08	14
45	7480	3080	1.08	17
60	17270	12100	1.12	43
90	31580	24060	1.78	81
120	34970	26570	1.19	90
180	36100	47450	1.40	93

Polymerisation condition: [eDEGA]:[I]:[$CuBr_2$]:[Me_6TREN] = 200:1:0.1:0.19. [a]Conversion measured by ^{1}H NMR spectroscopy. [b] THF eluent, linear PMMA standard.

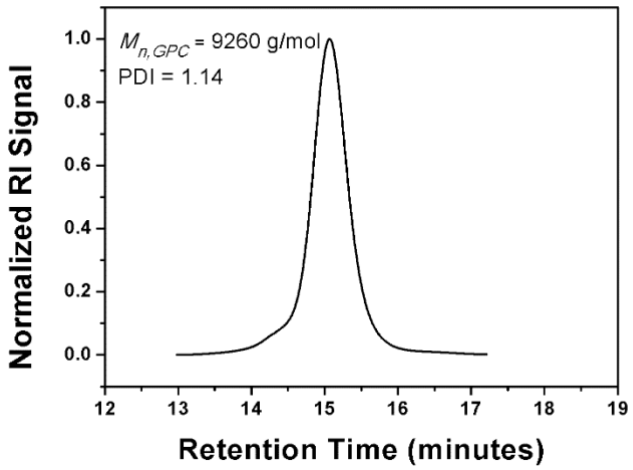


Figure S9: Obtained GPC trace for the purified star polymer of **P8.**

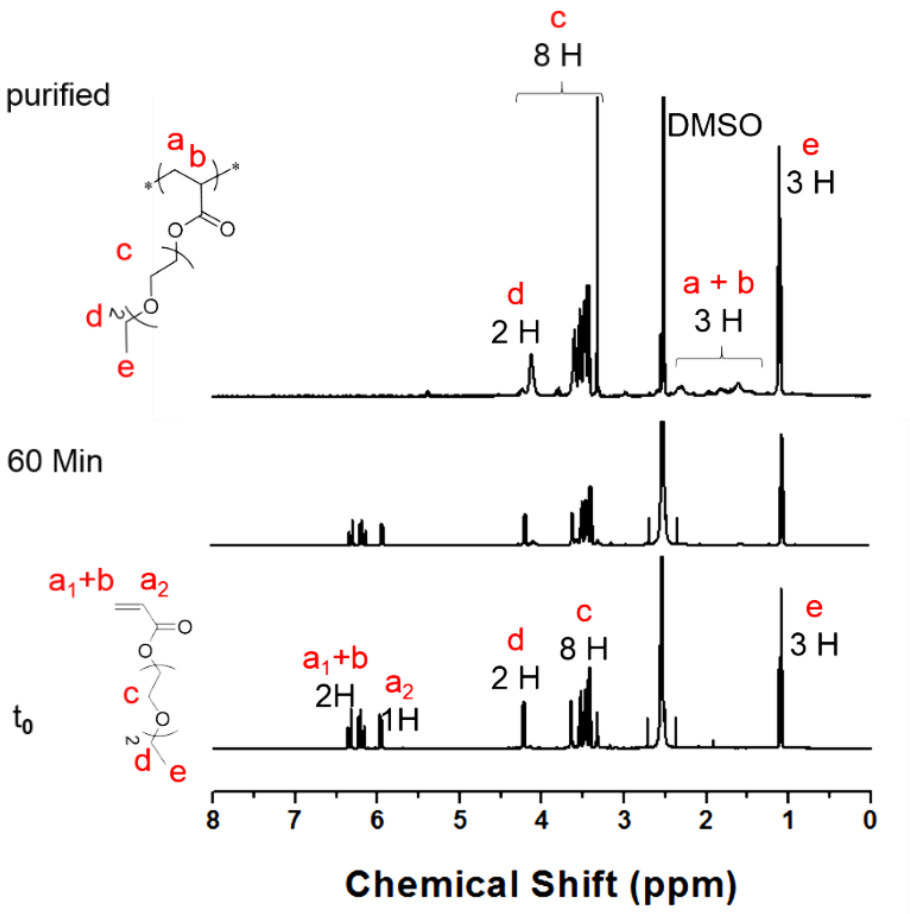


Figure S10: Overlay of the obtained ^{1}H NMR spectra for the star polymerisation of eDEGA using star initiator PETMP-Br$_4$ at time zero (**bottom**), after 60 minutes (**middle**) and star polymer after purification by dialysis. (**P8, top**).

3.2.3 General procedure for the native chemical ligation between P8 and Cys-ME

Cysteine methyl ester hydrochloride (14.5 mg, 8 eq.), TEA (20 µL, 16 eq.) and thiophenol (13 µL, 8 eq.) were dissolved in 0.5 mL dry DMF, which was added to a vial containing a solution of star polymer **P8** (100 mg, 1 eq.) in 0.5 mL dry DMF at 40°C. 20 µL of aliquots were removed periodically for the determination of molecular weight distribution *via* GPC.

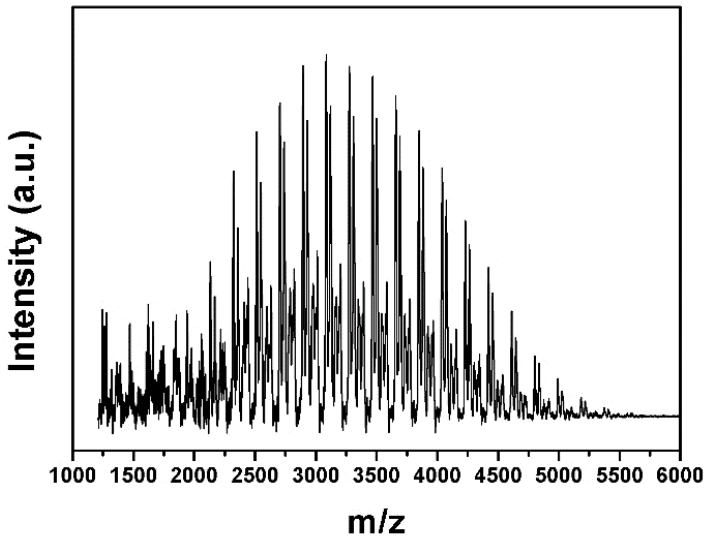


Figure S11: Obtained MALDI-ToF-MS spectrum, indicating full dissociation of **P8** to **P9**.

Table S4: Overview of the assigned MALDI-ToF-MS peaks and their corresponding calculated *m/z* values.

Peak$_{(DP)}$ – Chain end	**Formula**	**Calculated *m/z***	**Observed *m/z***
A_{14} – H	$C_{133}H_{237}NO_{57}SAg^+$	2900.45	2897.7
A_{15} – H	$C_{142}H_{253}NO_{61}SAg^+$	3087.55	3085.3
A_{16} – H	$C_{151}H_{269}NO_{65}SAg^+$	3275.65	3273.8
B_{14} – H	$C_{133}H_{237}NO_{59}SAg^+$	2931.43	2930.6
B_{15} – H	$C_{142}H_{253}NO_{63}SAg^+$	3119.54	3118.7
B_{16} – H	$C_{151}H_{269}NO_{67}SAg^+$	3307.64	3309.7
A_{14} – Br	$C_{133}H_{236}NO_{57}BrSAg^+$	2977.36	2978.5
A_{15} – Br	$C_{142}H_{252}NO_{61}BrSAg^+$	3165.46	3166.3
A_{16} – Br	$C_{151}H_{268}NO_{65}BrSAg^+$	3353.56	3354.2
B_{14} – Br	$C_{133}H_{236}NO_{59}BrSAg^+$	3009.35	3011.7
B_{15} – Br	$C_{142}H_{252}NO_{63}BrSAg^+$	3197.45	3199.9
B_{16} – Br	$C_{151}H_{268}NO_{67}BrSAg^+$	3385.66	3386.1