Poly(Boc-acryloyl hydrazide): The importance of temperature and RAFT agent degradation on its preparation.

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Abstract:
Poly(acryloyl hydrazide) is a versatile polymer scaffold readily functionalised through post-polymerisation modification with aldehydes to yield polymers for biological applications. However, its polymerisation is affected by nucleophilic degradation of the RAFT agent that leads to early termination, an issue often overlooked in the polymerisation of primary acrylamides. Here we report the effect of temperature on the RAFT polymerisation of N’-(tert-butoxycarbonyl)acryloyl hydrazide (1) and demonstrate that by carefully selecting this polymerisation temperature, a balance between rate of polymerisation and rate of degradation of the RAFT agent can be achieved. This way a greater control over the polymerisation process is achieved, allowing the synthesis of Boc-protected poly(acryloyl hydrazide) with higher degrees of polymerisation than those achieved previously, while still maintaining low dispersities. We believe our results should be of importance to those working on the RAFT polymerization of primary and secondary (meth)acrylamides and monomers with nucleophilic moieties.

Introduction:
Synthetic polymers are increasingly becoming an attractive means of interfacing biological systems via multivalent binding, displaying activity orders of magnitude higher than that of their monovalent components. Thus, polymers are now widely researched for biomedical applications including as antimicrobials, as drug and gene delivery vehicles, as biological sensors, or as "smart" biomaterials with anti-fouling properties. Highly functional polymers developed for specific applications generally involve the use of functional monomers which either already possess the final desired functionality, or have the capability of undergoing post polymerization modification to introduced the desired functionality. This latter approach can
greatly broaden the scope of chemical functionalities used. Post-polymerization modification has
normally relied on click chemistries, and has now been greatly expanded through the use of
oxime and hydrazone chemistry, reductive amination, and epoxide ring opening.

A common limitation when developing synthetic polymers for biomedical applications is the need
to screen large libraries of compounds which is costly and time consuming. In this regard,
acyrloyl hydrazide has been recently reported as a versatile platform for the synthesis and
screening of polymers for biomedical applications. Functional polymers are obtained by
simple incubation of poly(acryloyl hydrazide) with functional aldehydes, both under aqueous or
organic conditions, and this polymer has now been applied to the development of glycans,
ph sensitive drug-delivery, and nucleic acid delivery. In our laboratories poly(acryloyl hydrazide) was
prepared from Boc-protected precursor Boc-Px (Scheme 1) following deprotection with TFA.
Reversible Addition-Fragmentation (RAFT) polymerisation of N’-(tert-butoxycarbonyl)acryloyl hydrazide (1) resulted in a small library of polymers. However, control over the polymerisation was lost with increasing conversion and degree of
polymerisation, possibly as a result of degradation of the RAFT agent through intramolecular
nucleophilic attack. This degradation has been reported in the RAFT polymerisation of other
acylamide derivatives, including closely related methacryloyl hydrazide, with better control reported when the polymerisation is carried out at low temperatures. This side-
reaction is often overlooked in the polymerization of primary and secondary acryl- and
methacrylamides, and makes synthesising highly functional polymers from this type of
monomers inherently challenging. The need for greater control over these materials is more
significant when looking to understand better the nature of the structure-activity relationship
throughout post-polymerisation modification and biological screening.

Here, we report the effect of temperature and the decomposition rate of the initiator on
the polymerisation of N’-(tert-butoxycarbonyl)acryloyl hydrazide (1), as a route to optimise the
preparation of poly(acryloyl hydrazide). Polymerisations were carried out using 2,2’-azobis[2-(2-imidazolin-2-yl)propane]dihydrochloride (VA-044) as a low temperature initiator, so that the
rate of generation of radicals could be readily modified as a function of temperature. Our results

Scheme 1: RAFT polymerisation of N’-(tert-butoxycarbonyl)acryloyl hydrazide (1) and potential
degradation by-products.
suggest that while increasing the temperature increases the polymerisation rate, it also speeds up RAFT degradation and thus, loss of control. Conditions have been identified for which the polymerisation "outperforms" this side reaction and polymers with good control over molecular mass and dispersities (Đ) can be obtained. More importantly, these conditions allowed us to prepare Boc-Px with higher degrees of polymerisation and lower dispersities (Đ), not accessible with our previous conditions. We believe our results highlight the importance of balancing polymerisation kinetics and RAFT agent degradation in the polymerisation of monomers containing nucleophilic moieties such as acrylamides and methacrylamides. Moreover, this improved control over the polymerisation of Boc-protected poly(acyrloyl hydrazide) will be of value when degree of polymerisation and dispersity may underpin future applications.

**Experimental Section:**

Materials. 2-((Ethylthio)carbonothioyl)thio-2-methylpropanoic acid (CTA1) and N’-(tert-butoxycarbonyl)acryloyl hydrazide (1) were synthesised according to protocols described in the literature. 4-Cyano-4-(phenylcarbonothioylthio)pentanoic acid (CTA2) and cyanomethyl methyl(phenyl)carbamodithioate (CTA3) were purchased from Sigma-Aldrich® and used without any further purification. 2,2’-Azobis[2-(2-imidazolin-2-yl)propane] dihydrochloride (VA-044) was purchased from Fluorochem and used without further purification. All other chemicals were purchased from Sigma-Aldrich®, Fisher Scientific®, VWR® or Acros®, and used without further purification. All solvents were Reagent grade or above, purchased from Sigma-Aldrich®, Fisher Scientific® or VWR®, and used without further purification. Polymethylenacrylate standards were purchased from Agilent®.

**Characterisation:** Nuclear Magnetic Resonance (NMR) spectra were recorded on either a Bruker Avance III 300 MHz or a Bruker Avance III 400 MHz spectrometer. Chemical shifts are reported in ppm (units) referenced to the following solvent signals: dimethylsulfoxide (DMSO)-d₆, H 2.50. Gel Permeation Chromatography (GPC) was performed with a Shimadzu Prominence LC-20A fitted with a Thermo Fisher Refractomax 521 Detector and a SPD20A UV-vis Detector. poly(N’-(tert-butoxycarbonyl)acryloyl hydrazide) (Boc-Px) was analysed using 0.05 M LiBr in dimethylformamide (DMF) at 60 °C as the eluent, and a flow rate of 1 mL min⁻¹. The instrument was fitted with a Polymer Labs PolarGel guard column (50 × 7.5 mm, 5 μm) followed by two PLGel PL1110–6540 columns (300 × 7.5 mm, 5 μm). Molecular masses were calculated based on a standard calibration method using polymethylenacrylate standards.

**RAFT Polymerisation of N’-(tert-butoxycarbonyl)acryloyl hydrazide (1).** In a typical kinetic experiment, 2,2’-azobis[2-(2-imidazolin-2-yl)propane] dihydrochloride (VA-044) (11.7 mg, 0.036 mmol), 2-ethylthiocarbonothioylthio-2-methylpropanoic-acid (CTA) (40.3 mg, 0.18 mmol) and N’-(tert-butoxycarbonyl)acryloyl hydrazide (1) (1.666 g, 8.950 mmol) were dissolved in DMSO (10.0 mL) and a 100 μL sample was taken at this stage to calculate conversion (ρ). The solution vessel was sealed with a septum, securely fastened with electrical tape to maintain the
seal, and degassed by bubbling with argon for 25 minutes. Using a cannula, 1 mL of the solution was transferred to sealed glass vials containing stirrer bars, each degassed for 5 minutes. Vials were then left to react at a pre-set temperature (30-150 degrees °C) for the required amount of time. The reaction was stopped by allowing the tube to cool using a water bath and exposing it to air. 100 μL aliquots of each timepoint were taken at this stage to calculate conversion (ρ) and for GPC analysis. NMR and GPC analysis of each timepoint was carried out from the crude mixture. The natural logarithm of the inverse of the fractional concentration of monomer – \( \ln(M_0/M_t) \) – was plotted against time, and the data fitted using GraphPad Prism version 6.0 for Mac Os X, GraphPad Software, La Jolla California USA, www.graphpad.com. The in-built segmental line regression was used to fit the data to two intersecting lines. This model was used to identify when a change in the polymerisation kinetics was observed (\( t_{dead} \)).

**Results and discussion**

As reported, our initial efforts to optimise the polymerisation of Boc-protected acryloyl hydrazide 1 focused on reducing the temperature of the polymerisation.\(^{14}\) RAFT polymerisation of acrylamides and methacrylamides often suffer from cleavage of the RAFT agent through intramolecular addition-elimination of the weakly nucleophilic amides to the trithiocarbonate group (Scheme 1).\(^{25}\) In our previously reported conditions for the polymerisation of 1, a change in the rate of polymerisation was observed with increasing conversion (Figure 1A), which we associated with this degradation of the terminal trithiocarbonate in the growing chain. It has been proposed that reducing the polymerisation temperature would significantly reduce the rate of this side reaction.\(^{25}\) Thus, optimisation of the polymerisation was at that time carried out under the same conditions but using initiators with different 10 hour half-life decomposition temperatures (\( t_{10} \)) (Figure 1A). This way, rate of formation of radicals was kept as similar as possible for all polymerisations while reducing the temperatures to 50 °C (V-65) or 44 °C (VA-044). Despite the use of lower temperatures, in all cases, a change in the kinetics of the polymerisation was observed, although this change was not as obvious for the polymerisations performed at 44 °C (Figure 1A). To identify when this change in rate of polymerisation was occurring, the natural logarithm of the inverse of the fractional concentration of monomer – \( \ln(M_0/M_t) \) – was plotted against time, and the data fitted to a segmental line regression. This function fits the data to two different lines, before and after a breakpoint. In our case, we termed the breakpoint \( t_{dead} \) because we think that after this point, side reactions have a predominant effect on the kinetics of the polymerisation resulting in an increasing number of dead polymer chains. This change in kinetics was reflected on the relatively high dispersity in molecular mass (\( D = 1.38-1.95 \)) obtained for the polymers prepared with these conditions.\(^{14}\) Overall, no clear benefit from reducing the temperature was observed, with a \( t_{dead} \) of approximately 4 and 4.5 hours for polymerisations at 50 °C and 70 °C respectively. Interestingly, \( t_{dead} \) for the polymerisation performed at 44 °C was observed at approximately 2.5 h, which would suggest...
degradation was occurring faster at this temperature. This was not expected and may suggest that other mechanisms beyond the simple degradation of the RAFT agent may be at play.

Figure 1. A) Plot of fractional concentration of monomer $ln(M_0/M_t)$ vs time for polymerisations of N’-(tert-butoxycarbonyl)acryloyl hydrazide (1) performed at different temperatures. Conditions: $[M]=0.9M$, $[M]/[CTA]/[In]=100/1/0.2$. $A,4'$-Azobis(4-cyanovleric acid) (V-501) - circles, 2,2'-azobis(2,4-dimethylvaleronitrile) (V-65) - squares, and 2,2'-azobis[2-(2-imidazolin-2-yl)propane]dihydrochloride (VA-044) - triangles. Adapted with permission from Crisan, D. N.; Creese, O.; Ball, R.; Brioso, J. L.; Martyn, B.; Montenegro, J.; Fernandez-Trillo, F. Polym. Chem. 2017, 8 (31), 4576–4584 - Published by The Royal Society of Chemistry. B) For polymerisations carried out in 1A, effect of temperature on the time at which deviation from linearity for the plot of $ln(M_0/M_t)$ vs time is observed ($t_{dead}$), and the fractional concentration of monomer $ln(M_0/M_t)$ at this point.

Attempts to perform the polymerisation at an even lower temperature (30 °C) using VA-044 as the source of radicals resulted in a very long induction period followed by a short period of linear increase of the fractional concentration of monomer until a change in kinetics was again evident (Figure S1). The maximum conversion in this case was 50% - $ln(M_0/M_t) = 0.83$, worse than that observed for the polymerisations performed at higher temperatures.

In order to determine if degradation of the RAFT agent was indeed possible at low temperatures, we attempted to synthesise a small molecule analogue which mimicked an n=1 polymer (Scheme S1). To this end, 2-bromopropionic acid (2) was reacted with tert-butyl carbazate, and the resulting bromine derivative 3 reacted under standard conditions for the formation of the RAFT agent. $^1H$-NMR analysis of this reaction revealed a very complex mixture, where only traces of something that could resemble trithiocarbonate 4 could be identified (Figure S3). This observation was in line with our previous results, and suggested that hydrazide containing trithiocarbonates such as 4 were very amenable to intramolecular nucleophilic attack. Attempts to isolate this trithiocarbonate 4 were unsuccessful, with the main isolated product of this reaction being tentatively assigned to a mixture of the 5- and 6-membered rings in a 6:4 ratio (Figure S4).
Seeing how lowering the temperature had no beneficial effect on the kinetics of the polymerisation of 1, and a change in kinetics was still observed, we decided to explore the use of “Ultra-Fast” polymerisation conditions in an attempt to outrun the side reaction.\textsuperscript{30-32} Our hypothesis was that by using a low temperature initiator such as VA-044 at a significantly higher temperature (e.g. 100 °C) than the reported $t_{1/2}$ (44 °C), an increase in the concentration of radicals in solution would be achieved, and thus the concentration of propagating radicals would be higher with a greater number of chains growing at the same time, resulting in the synthesis of polymers with better control over the Mw and Đ. This methodology is particularly suitable for fast-propagating monomers such as acrylamides, and since the rate of polymerisation is directly proportional to the concentration of these propagating radicals (and the monomer concentration, \( R_p = k_p[M][P\cdot] \)), we postulated that running the polymerisation under these conditions could outperform the side reaction observed under standard RAFT polymerisation conditions. In a first attempt, the polymerisation conditions previously reported by us for the polymerisation of 1 (Figure 1)\textsuperscript{14} were modified so that the initiator used was VA-044 and the polymerisation temperature was 100 °C. A shorter polymer was targeted this time and, as expected, the polymerisation was very fast, reaching up to 70% conversion in less than five minutes (Figure 2A, CTA:VA-044 5:1 \( \bullet \)). The change in reaction rate could not be suppressed and was again evident, with a $t_{\text{dead}}$ of approximately 4.5 mins. Before $t_{\text{dead}}$ the polymerisation retained the features of a controlled polymerisation, with the molecular mass of the polymer directly proportional to the conversion and, comparable dispersities (Figure 2B, left) to those observed with our previous conditions.\textsuperscript{14}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{A) Plot of conversion ($\rho$) vs time and B) measured number average molecular mass (Mn) vs. conversion (●) and dispersity in molecular mass (Đ) vs conversion (○), for polymerisations of N-(tert-butoxycarbonyl)acryloyl hydrazide (1) performed with different CTA:VA-044 ratios. Conditions: [M]=0.9M, [M]/[CTA]=50/1. Mn and Đ calculated by GPC using 0.05 M LiBr in dimethylformamide (DMF) at 60 °C.}
\end{figure}

These results were promising and we therefore explored decreasing the concentration of initiator in our polymerisations, in an attempt to suppress termination, increase the number of chains growing from the RAFT agent and thus optimising the dispersities. However, while dispersities were decreased, reducing the concentration of initiator in these polymerisations resulted in slower reactions, with no effect observed in $t_{\text{dead}}$ (Figure 2A). As a result, the maximum
conversion obtained when the CTA:VA-044 ratio was increased to 10:1 or 15:1 (40% and 24% conversion respectively) was lower than in the previous case (70%).

We decided next to run the polymerisations at 150 °C, in an attempt to further increase the concentration of radicals during early stages of polymerisation, and thus the rate of propagation. However, these conditions not only resulted in lower conversions (Figure S8) but a colour change of the reaction mixture from yellow to dark brown, suggesting that thermal decomposition of the trithiocarbonate group was occurring.\(^{33}\) Thermal decomposition of the RAFT agent was confirmed via \(^{1}\)H-NMR where signals consistent with the \(\beta\)-elimination of the trithiocarbonate could be observed (Figure S9).\(^{33,34}\)

Having identified conditions to run the polymerisation of 1 at 100 °C, which resulted in similar conversions and dispersities to those previously reported, we decided to explore the use of these conditions to prepare polymers of higher Mw (Figure 3), which were harder to control using our previously reported method.\(^{14}\) Three different DPs were targeted (i.e. \([1]/[\text{CTA}] = 50, 100\) and 150), by maintaining the concentration of 1 and reducing the amount of RAFT agent and initiator used. As expected, this resulted in slower polymerisations while \(t_{\text{dead}}\) was still maintained at around 4.5 mins (Figure 3A). As a consequence, polymerisations targeting 100 and 150 monomer units only reached low conversions (~ 40% and 30% respectively). In any case, control over the molecular mass of the polymer was still observed during the first stages of the polymerisation, with the average molecular mass \((M_n)\) increasing linearly with time until the change in polymerisation rate was evident \((t_{\text{dead}})\) (Figure 3B). A clear shift towards lower retention time was observed in the gel permeation chromatograms when higher DPs were targeted, suggesting that, at least during the initial phase of the reaction, the polymerisation was maintaining features of a controlled radical polymerisation. Dispersities remained similar across the three targeted molecular masses which demonstrates an improvement compared to our previous conditions where the dispersity increased with increasing targeted DP.
Figure 3. A) Plot of conversion (ρ) vs time, B) fractional concentration of monomer \( \ln(M_0/M_t) \) vs time, and C) measured number average molecular mass (Mn) vs. time (top) and dispersity in molecular mass (D) vs time (bottom), for polymerisations of N'-(tert-butoxycarbonyl)acryloyl hydrazide (1) performed at 100 °C with different 1:CTA ratios. D) GPC chromatograms of the resulting polymers at the highest conversion obtained. Conditions: [M]=0.9M, [CTA]/[VA-044]=5/1. Mn and D calculated by GPC using 0.05 M LiBr in dimethylformamide (DMF) at 60 °C.

At this point, our results suggested that a compromise could be obtained between increasing the rate of propagation by increasing the polymerisation temperature, and delaying \( t_{\text{dead}} \) by reducing the polymerisation temperature. Therefore, we investigated polymerisations at intermediate temperatures (Figure 4). While a change in polymerisation rate was still evident for the new temperatures investigated (Figure 4A), higher conversions could be achieved for the polymerisation performed at 65 °C (90%) while the next highest conversions at 80 °C and 50 °C were 77% and 80% respectively (Figure 4B, ●). Temperature had a significant effect on the time at which a change in polymerisation rate was evident (\( t_{\text{dead}} \)), with this inflection point happening sooner as the temperature was increased (Figure 4B, ○).
Figure 4 A) Plot of fractional concentration of monomer $\ln(M_0/M_t)$ vs time for polymerisations of N’-(tert-butoxycarbonyl)acryloyl hydrazide (1) performed at different temperatures. B) Effect of temperature on the time at which deviation from linearity for the plot of $\ln(M_0/M_t)$ vs time is observed ($t_{\text{dead}}$ ($\bigcirc$), and the fractional concentration of monomer $\ln(M_0/M_t)$ at this point ($\bullet$). Conditions: $[M]=0.9M$, $[M]/[CTA]/[VA-044]=50/1/0.2$.

With encouraging results from the polymerizations at 65 °C, we set out to probe the livingness of the polymer before and after $t_{\text{dead}}$ and whether this $t_{\text{dead}}$ was an indication of degradation of the RAFT agent. To this end, we isolated and purified two polymerisations of 1, one that had been stopped at intermediate conversions ($\rho=47\%$, $t=30$ min), before $t_{\text{dead}}$ (Figure S5 A) and one that was stopped at maximum conversion ($\rho=85\%$, $t=120$ min), after $t_{\text{dead}}$ (Figure S5 B). As expected, Boc-P$_x$ isolated before $t_{\text{dead}}$ was able to undergo complete chain extension with further addition of 1 and initiator (Figure S5 A), thus demonstrating that at intermediate conversions the RAFT agent was still present in significant amounts. Boc-P$_x$ isolated after $t_{\text{dead}}$ showed no chain extension, instead showing a bimodal distribution of molecular mass and high dispersities (Figure S5 B) demonstrating that after $t_{\text{dead}}$ the RAFT group has been degraded. To probe if the RAFT agent degradation was temperature driven, we isolated and purified a second “living” Boc-P$_x$ at intermediate conversions ($\rho=52\%$, $t=30$ min) (Figure S6). This polymer was then heated for 90 minutes under standard polymerisation conditions, but this time without addition of 1 and initiator. We anticipated that heating the polymer this way should result in degradation of the RAFT agent, a hypothesis that was confirmed upon attempting to chain extend this terminated Boc-P$_x$. In this case, a shoulder was observed in the molecular mass and high dispersities indicating that the Boc-P$_x$ which had been subjected to further heating was “dead” (Figure S6). Additional evidence of the RAFT agent degradation was obtained from NMR spectroscopy, where the protons associated with both the R and Z end group of the polymer chain could be observed for the “living” Boc-P$_x$ whereas “dead” Boc-P$_x$ showed a loss of the Z group (Figure S7).

At this point, we decided to evaluate if further improvement could be achieved by optimising the RAFT agent used. For an effective RAFT process where the majority of the polymer chains grow at the same rate, the reactivity of the propagating chain and the stability of the polymer-RAFT intermediate should be optimised such that the addition to the C=S and subsequent fragmentation has a higher rate than propagation.28 Fast propagating monomers such as
acrylamides often benefit from RAFT agents which favour this radical addition to the C=S, such as trithiocarbonates like CTA1. However, we hypothesised that the bulky and electron-withdrawing nature of the Boc group in N’-(tert-butoxycarbonyl)acryloyl hydrazide (1) could have an impact on the reactivity of this monomer, and thus the Z-group in the RAFT agent could be modified. Two new RAFT agents were thus tested for their efficacy as chain transfer agents towards our monomer, each with Z substituents which stabilised or destabilised the polymer-RAFT intermediate, when compared to CTA1. Polymerisations were performed at 65 ºC and a DP of 200 was targeted. When CTA2 (Z = Ph) was used, a slower polymerisation was observed (Figure S10A, ◯), in agreement with the additional stabilisation of the intermediate radical provided by the phenyl group. However this decrease in rate of polymerisation also resulted in lower conversions, with no significant changes to $t_{\text{dead}}$ observed (Figure S10A, ◯). While these conditions showed some features of a controlled polymerisation, with low dispersities in molecular mass (Figure S10C, ◯), the resulting Mw for the polymers obtained were significantly higher than those where trithiocarbonate CTA1 was used (Figure S10B, ● for CTA1 and ◯ for CTA2). Alternatively, polymerisations performed with CTA3 (Z= N(Me)Ph) displayed faster reaction rates (Figure S10A, ◦) that we attribute to the low stability of the intermediate radical formed for dithiocarbamates. Unfortunately, these polymerisation conditions did not show any of the features of controlled polymerisations, with a decrease of Mw at high conversions (Figure S10B, ◦) and an increase in dispersity as the polymerisation proceeded (Figure S10C, ◦). In line with these observations, the Mw obtained this way for poly(N’-(tert-butoxycarbonyl)acryloyl hydrazide) was significantly higher than that obtained using CTA1 (Figure S10D, ●), despite both polymerisations reaching similar final conversions (Figure S10A).

Seeing how running the polymerisations at 65 ºC using CTA1 gave the highest conversions (90%) at $t_{\text{dead}}$ of all the conditions evaluated, we decided to target different degrees of polymerisation using these conditions (Figure 5). As before, targeting higher DPs resulted in slower rates of polymerisation, in particular for DP200 and DP300. While slower rates had a significant effect on the maximum conversion achieved (approx. 90%, 89%, 68% and 55% for DP 50, 100, 200 and 300 respectively), little effect was observed on the $t_{\text{dead}}$, with most polymerisations “stopping” after 1 h (Figure 5A).
Figure 5  A) Plot of fractional concentration of monomer \( \ln(M_0/M_t) \) vs time. B) Measured number average molecular mass (Mn) vs time (top) and dispersity in molecular mass (Đ) vs time (bottom), for polymerisations of N’-(tert-butoxycarbonyl)acryloyl hydrazide (1) performed at 65 °C with different 1:CTA ratios. C) GPC chromatograms of the resulting polymers at the highest conversion obtained. Conditions: [M]=0.9M, [CTA]/[VA-044]=5/1. Mn and Đ calculated by GPC using 0.05 M LiBr in dimethylformamide (DMF) at 60 °C.

Under these optimised conditions, the polymerisations retained features of a controlled polymerisation, with the molecular mass of the polymers increasing linearly with conversion, narrow dispersities in molar mass (Figure 5C) and good end group fidelity if isolated before \( t_{dead} \). In all cases, the dispersities obtained were similar or lower to those reported previously.\(^\text{14}\) This was particularly the case when targeting DPs of 100 and 200 with dispersities of <1.4 being observed at maximum conversion.

**Conclusion**

Here we have demonstrated the role of temperature and RAFT agent degradation in the polymerisation of N’-(tert-butoxycarbonyl)acryloyl hydrazide (1). Our results highlight that the polymerisation of acrylamides via RAFT can be severely hampered by the degradation of the chain transfer agent and that, under some circumstances, this degradation cannot be eliminated but rather outperformed if the rate of polymerisation is tuned. We demonstrate that by using a low-temperature initiator such as VA-044, optimal polymerisations conditions can be achieved at 65 °C. This way, poly(N’-(tert-butoxycarbonyl)acryloyl hydrazide)s with high degrees of polymerisation could be obtained while still maintaining low dispersities. Finally, we demonstrate that for our system, no benefit is obtained when trithiocarbonates are replaced with dithioesters or trithiocarbamates, as the chain transfer agents.

**Author contributions.**

PFT, OC and PA designed the work. OC, PA GS and AR performed all the experimental work. OC and PFT analysed the data and wrote the paper, with all other authors contributing to the final version of the manuscript.
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Poly(Boc-acryloyl hydrazide): The importance of temperature and RAFT agent degradation on its preparation

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Figure S1. Plot of ln(M₀/Mₜ) vs time for the polymerisation of N’-(tert-butoxycarbonyl)acryloyl hydrazide (1) at 30 °C. Conditions: [M]=0.9M, [M]/[CTA]/[VA-0 44]=50/1/0.2.
Small molecule analogue of a DP= 1 of N’- (tert-butoxycarbonyl)acryloyl hydrazide (1).

Scheme S1: Attempted route for the synthesis of a DP= 1 analogue of N’- (tert-butoxycarbonyl)acryloyl hydrazide (1).

**tert-butyl 2-(2-bromopropanoyl)hydrazine-1-carboxylate (3):** 2-Bromopropionic acid (2) (10 g, 59.9 mmol) and tert-butyl carbazate (6.56 g, 49.6 mmol) were dissolved in a 2:1 mixture of water/THF (180 ml). N-(3-Dimethylaminopropyl)-N’-ethylcarbodiimide hydrochloride (13.3 g, 69.5 mmol) was added in portions to the solution over 15 minutes and the mixture was left stirring for 3h at room temperature. The solution was extracted into EtOAc (3 x 60 ml) and a basic work-up performed with NaCO₃ (3 X 60 ml). The organic layer was further washed with water (2 x 60 ml), dried with Na₂SO₄, filtered and the solvent removed under reduced pressure to leave a white solid. This solid was then recrystallised using ethyl acetate to afford white crystalline material which was washed with ice cold diethyl ether and dried under reduced pressure (8.9 g, 64 %): ¹H NMR (300MHz, DMSO-d₆) δ (ppm) 9.9 (s, 1H), 9.0-8.3 (s, 1H), 4.45 (q, 1H), 1.65 (d, 3H), 1.38 (s, 9H).

**tert-butyl 2-((ethylthio)carbonothioyl)thio)propanoyl)hydrazine-1-carboxylate (4) (not isolated):** Ethanethiol (0.49 ml, 6.59 mmol) was added to a suspension of K₃PO₄ (1.4 g, 6.59
mmol) in acetone (20 ml) and was left stirring at room temperature for 10 minutes. CS$_2$ (1.09 ml, 6.59 mmol) was then added and the reaction mixture was left for a further 10 minutes. tert-butyl 2-(2-bromopropanoyl)hydrazine-1-carboxylate (1) (1.6 g, 5.99 mmol) was added in one portion and the mixture left to react for 13 hours. The solvent was then removed under reduced pressure and HCl (100 ml, 1 M) was added to the crude of the reaction. The resulting mixture extracted into DCM (2 x 100 ml). The organic layer was then washed with water (2 x 100 ml) and brine (2 x 100 ml), dried with Na$_2$SO$_4$, filtered and the solvent removed under reduced pressure. The resulting orange oil was purified by column chromatography using a 7:3 ratio of diethyl ether and hexane, then dried under reduced pressure to leave a viscous orange liquid (0.12 g, 7 %) which consisted of two compounds, none of which is the title compound. a; $^1$H NMR (300MHz, CDCl$_3$) δ (ppm) 10.3-9.7 (1H, s, NH), 4.66 (q, 1H), 1.58 (d, 3H), 1.44 (s, 9H) and b; $^1$H NMR (300MHz, CDCl$_3$) δ (ppm) 10.3-9.7 (1H, s, NH), 4.73 (q, 1H), 1.59 (d, 3H), 1.44 (s, 9H).

Figure S3: A) $^1$H NMR (300 MHz, DMSO) spectrum of 2-((ethylthio)carbonothioyl)thio-2-methylpropanoic acid (CTA1). B) $^1$H NMR (300 MHz, CDCl$_3$) spectrum of tert-butyl 2-(2-bromopropanoyl)hydrazine-1-carboxylate (3). C) $^1$H NMR (300 MHz, CDCl$_3$) spectrum of the reaction of ethanethiol with carbon disulfide and tert-butyl 2-(2-bromopropanoyl)hydrazine-1-carboxylate (3).
Figure S4: $^1$H NMR (300 MHz, CDCl$_3$) spectrum of the main fraction isolated following the reaction of ethanethiol with carbon disulfide and tert-butyl 2-(2-bromopropanoyl)hydrazine-1-carboxylate (3).

Figure S5: Left: GPC traces (DMF LiBr 0.05M) of “living” Boc-Px after ($t=30$ min) and subsequent chain extension with (1)($t=30+60$ min). Right: “dead” Boc-P$_x$ ($t=120$ min) and subsequent inability to chain extend with (1) ($t=120+60$ min) (B).
Figure S6: GPC traces (DMF LiBr 0.05M) of isolated “living” Boc-Pₙ after (t=30 min) after further heating (60 °C t=90 min), and subsequent inability to chain extend with (1) (t=30+60 min).
Figure S7: Top: $^1$H NMR (300 MHz, CDCl$_3$) of “living” Boc-Px after polymerisation reaction was stopped after 30 minutes, before full conversion (47%). Bottom: $^1$H NMR (300 MHz, CDCl$_3$) of “dead” Boc-Px after polymerisation for 120 minutes to maximum conversion (85%).
Figure S8: Plot of $\ln(M_0/M_t)$ vs time (A) and conversion ($\rho$) vs time (B) for the polymerisation of N’-(tert-butoxycarbonyl)acryloyl hydrazide (1) at 150 °C. Conditions: [M]=0.9M, [M]/[CTA]/[VA-044]=50/1/0.2.

Figure S9: $^1$H NMR (300 MHz, CDCl$_3$) spectrum showing vinyl region at varying time points in the polymerisation of N’-(tert-butoxycarbonyl)acryloyl hydrazide (1) at 150 °C. Conditions: [M]=0.9M, [M]/[CTA]/[VA-044]=50/1/0.2. New vinyl protons can be observed from 7 minutes, suggestive of β-elimination products.
Figure S10: A) Plot of fractional concentration of monomer $\ln(M_0/M_t)$ vs time; B) measured number average molecular mass (Mn) vs. time; C) dispersity in molecular mass (Đ) vs time; and D) GPC chromatograms of the resulting polymers at the highest conversion obtained, for polymerisations of N’-(tert-butoxycarbonyl)acryloyl hydrazide (1) performed at with different chain transfer agents. Conditions: [M]=0.9M, [1]/[CTA]=200/1, [CTA]/[VA-044]=5/1, 65 ºC. Mn and Đ calculated by GPC using 0.05 M LiBr in dimethylformamide (DMF) at 60 ºC.