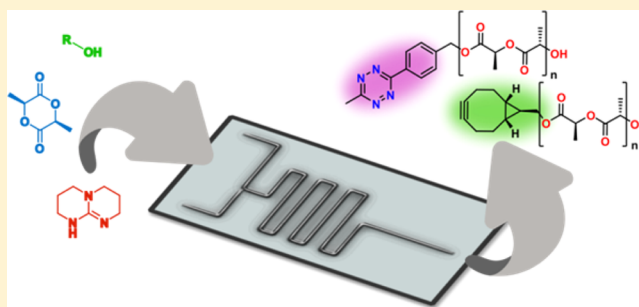


## Clickable Polylactic Acids by Fast Organocatalytic Ring-Opening Polymerization in Continuous Flow

Sebastiaan A. van den Berg,<sup>†</sup> Han Zuilhof,<sup>\*,†,‡</sup> and Tom Wennekes<sup>\*,†,§</sup><sup>†</sup>Laboratory of Organic Chemistry, Wageningen University, Dreijenplein 8, 6703 HB, Wageningen, The Netherlands<sup>‡</sup>Department of Chemical and Materials Engineering, King Abdulaziz University, Jeddah, Saudi Arabia<sup>§</sup>Department of Chemical Biology and Drug Discovery and Utrecht Institute for Pharmaceutical Sciences and Bijvoet Center for Biomolecular Research, Utrecht University, Universiteitsweg 99, 3584 CG Utrecht, The Netherlands

## S Supporting Information

**ABSTRACT:** The use of microreactor technology for the ring-opening polymerization of L-lactide catalyzed by 1,5,7-triazabicyclo[4.4.0]dec-5-ene allows for rapid optimization of reaction parameters (reaction temperature and residence time). At moderate catalyst loading, good control over the polymerization is demonstrated by high conversion of monomer (>95%) and low polydispersity (<1.3) at residence times as low as 2 s. This metal-free, organocatalytic route yields well-defined poly(lactic acid) in continuous flow and by using bicyclononyne- and tetrazine-containing initiators gives access to poly(lactic acid) amenable to click chemistry.



## ■ INTRODUCTION

The use of continuous flow microreactors is an attractive method to improve the control over polymerization reactions due to the exquisite control over reaction parameters. Nevertheless, microreactor technology is still sparsely used in polymer chemistry.<sup>1</sup> Recent advances have led to microstructured devices where extremely rapid mixing and precise control over temperature are possible. The excellent heat transfer and mixing properties are ideally suited for e.g. exothermic polymerizations.<sup>1</sup> In some cases, continuous flow allows the use of far less stringent conditions and simpler experimental procedures compared to batch, allowing higher temperatures and potentially dangerous reaction conditions to be used safely.<sup>2,3</sup> Because of the small reaction volumes and short reaction times employed, fast screening of reaction conditions is possible with minimal reagent use.<sup>4–6</sup> Once identified, optimal conditions can also be scaled up more reliably compared to batch as the advantageous mixing and heat transfer characteristics are largely maintained in larger flow setups.

We are interested in the organocatalyzed ring-opening polymerization of L-lactide to produce poly(lactic acid) for biomedical and other applications and reasoned that this polymerization could benefit from the use of microreactors (see Figure 1).

To date, the use of continuous flow techniques has been reported for several types of polymerizations, including reversible addition–fragmentation chain-transfer (RAFT),<sup>9,10</sup> atom-transfer radical (ATRP),<sup>11,12</sup> and living anionic<sup>2,3</sup> as well as ring-opening polymerization.<sup>4,13,14</sup> Depending on the type of polymerization, improvement over batch comes from improved

heat transfer or faster mixing. Iida et al. studied living anionic styrene polymerizations, initiated with *sec*-butyllithium, in continuous flow.<sup>3</sup> Interestingly, they observed a dependence of the resulting molecular weight distribution on the channel geometry, especially when performing polymerizations that generated a highly viscous reaction medium. Tonhauser and co-workers also studied the living anionic polymerization of styrene.<sup>2</sup> The anionic polymerization of styrene in a tubular steel reactor yielded very fast reactions (15 s in flow vs 3 h in batch) at the expense of a broadened molecular weight distribution. Precise *online* end-capping of the resulting polymers was done by introducing functionalized epoxides into the microreactor via a separate channel. Natalello and co-workers demonstrated the living anionic polymerization of styrene and 2-vinylpyridine (2VP). By using special micro-mixers in continuous flow, they were able to synthesize well-defined, high-molecular-weight PS and P2VP (polydispersity index (PDI)  $\approx$  1.05,  $M_n$  up to 149 000 g/mol for PS) at room temperature vs  $-78$  °C in batch.<sup>14</sup>

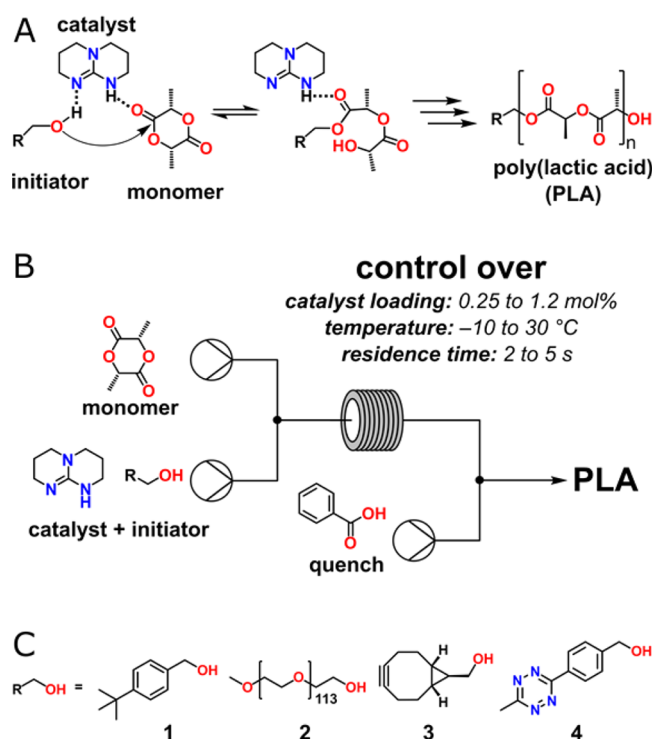
Hornung et al. performed the first solution-phase RAFT polymerization of acrylates and acrylamides in continuous flow on a multigram scale.<sup>9</sup> Batch and flow modes of operation performed similarly, yielding in each case polymers with molecular weights up to 25 000 g/mol and polydispersities between 1.09 and 1.16.

Baeten and co-workers showed the elegant use of microreactors to quickly screen the cationic ring-opening polymer-

Received: November 23, 2015

Revised: February 13, 2016

Published: March 3, 2016



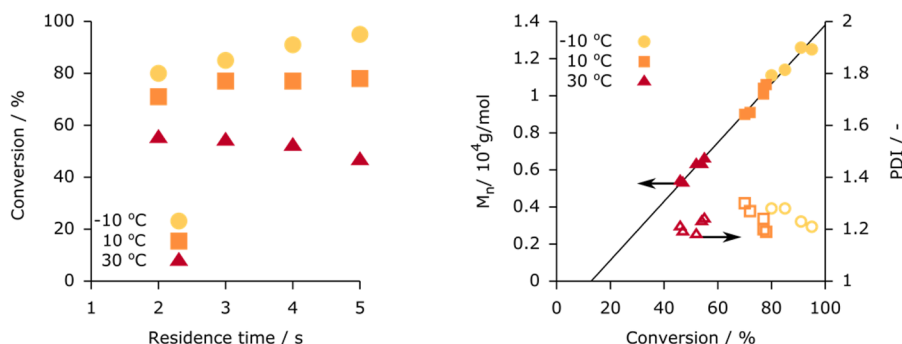
**Figure 1.** (A) TBD-catalyst mode of action in the ring-opening polymerization of L-lactide: hydrogen bonding increases nucleophilicity<sup>7,8</sup> of alcohol initiator. (B) The continuous flow organocatalytic polymerization of L-lactide is performed in a commercially available microreactor setup using a 1  $\mu$ L internal volume glass microreactor chip. (C) Alcohol initiators in current study: 4-*tert*-butylbenzyl alcohol 1, PEG macroinitiator 2, *exo*-BCN-alcohol 3, and tetrazine 4.

ization of 2-oxazoline at temperatures well above the boiling point of the solvent. Despite its sensitivity to water, the polymerization in the microreactor proceeded rapidly and with minimal side-reactions. By linking two microreactor chips together, di- and triblock copolymers could be synthesized.<sup>4</sup> Hawker et al. used a light-initiated controlled radical polymerization reaction catalyzed by an iridium complex to generate poly(methyl methacrylate) in flow.<sup>15</sup> The use of heterogeneous catalysts, on the other hand, had a negative influence on the control over the ATRP of methacrylate as Shen and co-workers showed. Using a solid-supported Cu<sup>I</sup> catalyst in flow gave a broader molecular weight distribution than when the same catalyst was used in batch. Because of the increase in viscosity, polymer chains are increasingly hindered from entering the catalytic sites on the solid support. As a consequence, these chains are not deactivated fast enough, leading to uncontrolled polymer growth.<sup>11</sup> Poor PDI values are, however, not intrinsic to in-flow polymer forming reactions, as e.g. elegantly shown by Leibfarth and co-workers, who used flow-iterative exponential growth to generate unimolecular oligomers (up to the octamer) via the copper-catalyzed Huisgen 1,3-dipolar cycloaddition. While strictly not a polymerization reaction, the work of Leibfarth et al. generates oligomers with extremely low PDI (<1.01 vs PS standards), and the synthesis was readily scaled to 2.75 g/h.<sup>16</sup>

Here we show that microreactors are also applicable to another type of homogeneously catalyzed polymerization reaction, namely the ring-opening polymerization of L-lactide to poly(lactic acid) (PLA), a popular biodegradable and biocompatible polyester derived from renewable resources.

PLA is widely and increasingly applied in packaging, disposable bottles, and biomedical applications with an annually increasing production (185 kilotons in 2013).<sup>17,18</sup> Low-molecular-weight PLA can be synthesized from a condensation reaction of 2-hydroxypropionic acid (lactic acid); however, the ring-opening polymerization (ROP) of cyclic lactide monomers gives access to the practically more useful polymers of high molecular weight.<sup>17</sup> The industrial synthesis of PLA currently relies heavily on metal catalysts, such as tin(II) octoate, at elevated temperatures. Other catalysts for the ROP of lactide have been investigated, including phosphines,<sup>19</sup> 4-(dimethylamino)-pyridine,<sup>20</sup> organophosphates,<sup>21</sup> and *N*-heterocyclic carbenes.<sup>22</sup> A promising alternative is the organocatalyzed polymerization of L-lactide using amidine and guanidine bases.<sup>23–25</sup> Pratt et al. showed that 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD) is a very potent organocatalyst for the ROP of cyclic esters.<sup>26</sup> The major drawback of TBD, however, is that besides catalyzing the ring-opening of cyclic esters, it also catalyzes the transesterification of the polyester backbone (see Figure 1).<sup>26,27</sup> This leads to an undesired increase in polydispersity. In the presence of an excess of an alcohol, TBD can even be used to depolymerize PLA, generating esters of lactic acid.<sup>28</sup> To suppress transesterification, the polymerization is therefore quenched by manual addition of benzoic acid after a defined short reaction time. Other, less active catalysts such as DBU and thiourea derivatives have been found to work better for PLA polymerization in batch. Continuous flow, however, presents an opportunity to utilize the high activity of TBD while limiting transesterification by virtue of the excellent control over reaction parameters. Furthermore, TBD-catalyzed lactide polymerizations in batch typically take less than 60 s to reach full conversion, even at low catalyst loadings (down to 0.1 mol % relative to monomer).<sup>26</sup> Using this procedure, Pratt et al. were able to obtain PLA with molecular weights up to 63 000 g/mol and PDIs of 1.1–1.2.<sup>26</sup> While highly attractive to further investigations, the sensitivity to water currently requires this polymerization to be performed in a glovebox.<sup>23,26</sup> Furthermore, quenching of the polymerization by manual benzoic acid addition after the very short reaction times is not very reproducible due to difficulties in timing and mixing. Taken together, this limits the practical applicability of this method. We reasoned that the TBD-catalyzed ROP of L-lactide is an ideal candidate for optimization in continuous flow microreactors, allowing for more control over this reaction, without the need for a glovebox, and a better insight into the parameters controlling the polymerization. In microreactors, the mixing of reagents is fast (on the order of milliseconds) and the residence time before addition of a quenching agent can be precisely tuned. Finally, the temperature of the reaction can be adjusted quickly and maintained accurately. This allows for fast screening of reaction conditions with high reproducibility and a practical continuous production of PLA outside of the glovebox (see Figure 1).

In the light of biomedical applications of PLA and our ongoing research in this area, we were interested in obtaining PLA-based materials that could be functionalized efficiently with (bio)molecules through the use of click chemistry.<sup>29–31</sup> PLA and PEG–PLA (co)polymers have been shown to assemble into nanoparticles, which would enable their use as drug carriers by conjugating small bioactive molecules to the chain end.<sup>32,33</sup> Facile functionalization of a polymer chain end can be achieved by introducing a “clickable” moiety such as an alkyne or azide. The use of copper-catalyzed azide–alkyne



**Figure 2.** Left: conversion of monomer at 0.25 mol % catalyst loading vs residence time. Right:  $M_n$  vs conversion follows a straight line. Catalyst loading: 0.25 mol %. Closed symbols denote  $M_n$ ; open symbols denote PDI.

cycloaddition (CuAAC) click chemistry for polymer functionalization has indeed been used extensively.<sup>34</sup> The development of a metal-free, organocatalytic route toward PLA, however, begs for a copper-free alternative. The strain-promoted azide-alkyne cycloaddition (SPAAC) reaction and inverse electron demand Diels–Alder (IEDDA) reaction are good candidates for metal-free alternatives. SPAAC uses the inherent ring strain in cyclooctynes to react with azides in a copper-free manner.<sup>35</sup> One way to introduce this cyclooctyne moiety is by postfunctionalization of the polymers, as shown by Boons and co-workers for poly(ethylene glycol)-*block*-poly( $\epsilon$ -caprolactone);<sup>36</sup> this, however, introduces extra steps in the synthesis and would require postpolymerization purification of these materials. Therefore, we hypothesized that cyclooctyne-derived alcohol **3** and tetrazine-derived alcohol **4** could be used as initiators, as this would allow for full incorporation of the clickable moiety in each polymer chain. There is some precedent for the use of strained alkynes, such as 4-dibenzocyclooctynol, and tetrazines as clickable initiators for ROP, ATRP, and RAFT polymerizations.<sup>37–40</sup>

In this paper, we demonstrate the facile metal-free synthesis from L-lactide of well-defined PLA with a low PDI in continuous flow without the need for a protective atmosphere during the polymerization. The polymerization is performed with unprecedented short residence times, with optimal values as low as 2 s. Furthermore, we show for the first time the direct synthesis of SPAAC-ready PLA, initiated from the cyclooctyne-containing bicyclo[6.1.0]nonyne-derived alcohol **3**, and its click reaction with a set of azides. In addition, we also prepared the first example of tetrazine-containing PLA from tetrazine-derived alcohol **4** that can readily react with norbornenes and other tetrazine-click compatible reaction partners such as strained alkynes and alkenes.<sup>40</sup> Scaling out in the microreactor chip under optimized reaction conditions leads to 200 mg of polymer per hour, which is easily purified by precipitation and can potentially be scaled up in industrial flow reactors. As toxic metals are completely avoided in the synthesis, our approach is ideally suited for synthesis of functional PLA materials for use in biomedical and other applications.

## RESULTS AND DISCUSSION

A series of experiments were performed in a continuous flow microreactor to study the effects of catalyst loading, residence time, and temperature on the conversion of monomer and molecular weight distribution of the resulting PLA. We reasoned that a 1  $\mu\text{L}$  microreactor chip would allow for the right residence time window and the quick screening of these reaction conditions. Four catalyst loadings were investigated

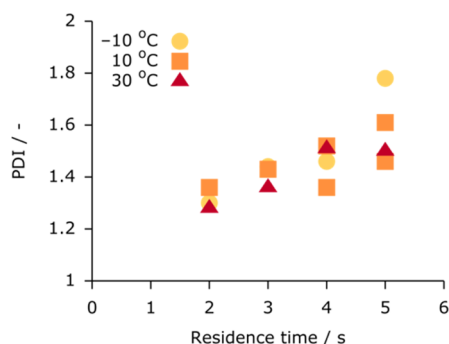
(1.2, 0.5, 0.25, and 0.1 mol % with respect to monomer). The ratio between monomer and initiator was kept constant at 80:1, thus targeting a degree of polymerization (DP) of 80 unless noted otherwise. The catalyst can be rapidly quenched by on-chip mixing of benzoic acid into the reaction stream at the end of the reaction zone, allowing precise control over the residence time. Analysis by  $^1\text{H}$  NMR yielded conversion and DP, while GPC was used to determine  $M_n$  (vs PS standards) and PDI. End-group fidelity was determined by MALDI-ToF-MS (see Figure S28). Quantitative conversion of monomer was easily achieved within seconds using 1.2% of catalyst with respect to monomer, whereas the conversion was typically less than quantitative at catalyst loadings of 0.1–0.5 mol %.

For example, with 0.5% catalyst the monomer conversion was 91–100% with concomitant polydispersities between 1.24 and 1.52. A catalyst loading of 0.25 mol % was chosen to study the influence of temperature and residence time on the polymerization (Figure 2). Three different temperatures (−10, 10, and 30 °C) and four different residence times (2, 3, 4, and 5 s)<sup>41</sup> were examined. At 0.25 mol % catalyst loading, the conversion ranged from 45 to 95% (see Figure 2, left). This broad range allowed us to clearly visualize the trends in conversion caused by the reaction parameters for a 0.25 mol % catalyst loading, as shown in Figure 2 (see Supporting Information for tabulated data including other catalyst loadings). In these experiments the standard deviation in the given conversions is typically 5% or less (based on triplicate experiments; see Figure S25), indicating our continuous flow system can accurately map the trends of this polymerization.

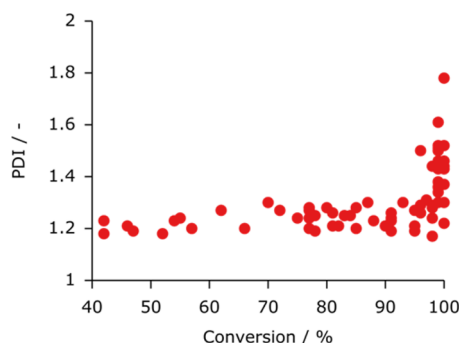
We investigated the polymerization further by measuring the conversion at nine different temperatures at a fixed residence time (−10 to 30 °C, every 5 °C; 4 s) and calculating  $\Delta H^0$  from the Van't Hoff plot (see Figure S13). This value was found to be −20.8 kJ/mol and is in good agreement with the value of  $\Delta H_p$  of −22.9 kJ/mol as previously reported by Duda et al.<sup>42</sup> This indicates that at this low catalyst loading a thermodynamic equilibrium is reached even after these short 2–5 s residence times.

At higher catalyst loading (0.5 mol % with respect to monomer and up), the conversion of monomer is near-quantitative under all tested conditions. Monomer conversions reached  $\geq 98.5\%$  under all investigated conditions when using a fixed catalyst loading of 1.2 mol %, even at the shortest 2 s residence time. Residence times beyond 2 s only resulted in broadening of the molecular weight distribution (see Figure 3).

This is in line with previous findings by Lohmeijer et al., who found that transesterification of the polymer backbone becomes more pronounced at high conversions (see Figure 4).<sup>20</sup> The



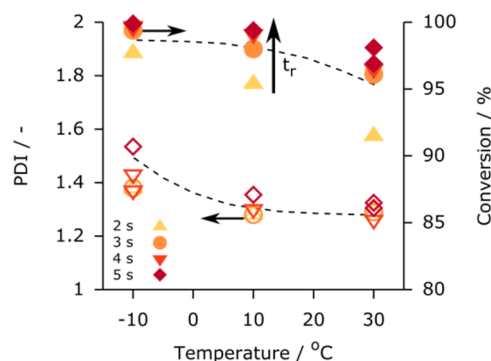
**Figure 3.** Molecular weight distribution due to transesterification of the polymer backbone versus residence times. Catalyst loading 1.2 mol %; conversion at all residence times  $\geq 98.5\%$ .



**Figure 4.** Plot of PDI versus conversion for all experiments at a targeted DP of 80.

same dependence of PDI vs conversion is found for the polymerization of L-lactide using tin(II) octoate at 190 °C, which is also attributed to transesterification.<sup>43</sup> Despite this trend toward increased PDI at the high catalyst loading of 1.2 mol %, the use of the microreactor allowed for rapid screening toward optimal reaction parameters. A residence time of 2 s and a reaction temperature of 30 °C led to polymers with an acceptable PDI of 1.28 and  $M_n$  of 14 500 g/mol. This shows that control over the residence time, and thus the exact time when the catalyst is quenched, plays a crucial role in controlling this polymerization.

Broadening of the polymer weight distribution was less pronounced at lower catalyst loadings because the transesterification process is slower at the less than quantitative conversion observed at these loadings and therefore has less influence. A transition can be seen in the case of 0.5 mol % catalyst loading. The increase in residence time leads to the conversion approaching 100% at -10 °C and a concomitant increase in PDI from 1.2 to 1.5 going from 2 to 5 s residence time. For 10 and 30 °C, where the conversions are 99% and 91–96%, respectively, the PDI decreases with respect to that observed at -10 °C, independent of residence time (see Figure 5). We tentatively attribute the typically higher polydispersities observed at lower temperatures to the higher conversions reached at these temperatures, which allows more transesterification to take place. Nevertheless, because reaction conditions could be rapidly screened, we determined that at a catalyst loading of 0.25 mol % the optimal conditions of a 5 s residence time at -10 °C achieve a 95% conversion of monomer. This gave PLA with an  $M_n$  of 12 500 g/mol and a PDI of 1.21 and demonstrates good control over the polymerization in the microreactor. This is especially note-



**Figure 5.** Monomer conversion (closed symbols) and resulting PDI values (open symbols) of TBD-catalyzed PLA formation vs reaction temperature (0.5 mol % TBD). As residence time goes up ( $t_r$ ), so does monomer conversion (closed symbols). This trend is visible at all three temperatures; however, the overall conversion goes down as temperature increases. Finally, PDI values go down as temperature increases. Dashed lines only serve as a guide to the eye.

worthy as all these results are obtained without the need to perform the polymerization under an inert atmosphere, where this was deemed essential for all batch-based, TBD-catalyzed PLA polymerizations.<sup>20,26</sup>

Higher degrees of polymerization (DP 160 and 240) can be obtained with the microreactor flow setup by adjusting the initiator-to-monomer ratio. The appropriate solutions of monomer (concentration kept at 1.6 M) and initiator were prepared for each targeted DP, using 0.01 and 0.0067 M initiator for DP 160 and 240, respectively. For a DP of 160, at 0.6 mol % TBD relative to monomer, the polymerization showed very similar trends as for the polymerization targeting a DP of 80 at 0.5 mol % TBD. Conversion increased with increasing residence time (see Figure S8), and the PDI of the resulting polymers was  $\approx 1.2$ . Full conversion was found at a residence time of 5 s and temperature of -10 °C. The resulting PLA had a  $M_n$  of 22 000 g/mol, a PDI of 1.22, and analysis by <sup>1</sup>H NMR revealed the DP to be 169.

For a targeted DP of 240, the monomer concentration was kept constant at 1.6 M and the initiator concentration was lowered to 0.0067 M. Controlled variation of the residence time and catalyst loading showed that at 1.2 mol % TBD full conversion was reached after 3 s at 10 °C ( $M_n$  38 500 g/mol, PDI 1.29). Attempting to polymerize the monomer toward a DP of 240 at a lower catalyst loading (0.75 mol %) resulted in a 3 s residence time and -10 °C as optimal conditions: conversion was 87% and PDI was 1.17 (see Figure S9).

**Batch Polymerizations Give Lower Conversion.** Next we assessed how the polymerization performed in batch mode under the optimized conditions identified in the flow setup. Using oven-dried vials under nitrogen flow, the polymerization was conducted at room temperature (20 °C) with 0.5 mol % catalyst loading and was stopped after a reaction time of 3 s by manual addition of a benzoic acid solution. The resulting reaction mixtures were analyzed by <sup>1</sup>H NMR and GPC, showing  $49 \pm 6\%$  conversion and PDI values of  $\approx 1.2$ . Using the same stock solutions, the polymerization was performed in flow (at a temperature of 20 °C), giving 91% conversion at a residence time of 3 s and a similar PDI (1.2). This shows that greater control and conversion is achieved in flow without the need for a glovebox. A 30 min scale out under these conditions at 10 °C, followed by precipitation in diethyl ether,

centrifugation, and drying of the crude polymer, resulted in 64 mg of a white, fluffy polymer (93% yield, DP = 76, PDI 1.19). This extrapolates to >120 mg of polymer per hour, demonstrating that even a microreactor can already produce useful amounts of well-defined polymer for research, whereas this can in principle be scaled up for commercial application in a mesoscale flow reactor.<sup>44</sup>

**Monomer Conversion Increases upon Lowering the Reaction Temperature.** Interestingly, the general trend at these lower catalyst loadings (i.e., <0.25 mol %) is that the conversion increases with decreasing reaction temperatures (see Figure 2, top). This phenomenon is also observed by Martello et al. for the polymerization of a lactone with TBD.<sup>45</sup> In the case of ring-opening polymerizations, the entropy decreases going from monomer to polymer.<sup>42</sup> The reaction is therefore driven by the exothermic enthalpic contribution from the release of ring strain. L-Lactide exhibits only moderate ring strain, which leads to an equilibrium between polymer and monomer. Duda et al. have reported that the monomer equilibrium concentration,  $[M]_{eq}$ , is higher at higher reaction temperatures, which matches our findings.<sup>42</sup> This has also been found for other polymerizations of low-strain monomers; i.e., higher conversions are obtained at lower temperatures.<sup>46,47</sup> Indeed, using the values for  $\Delta H_p$  and  $\Delta S_p^0$  as determined by Duda et al. for PLA formation from L-lactide, the calculated Gibbs free energy of the polymerization,  $\Delta G$ , is  $-12.1$  kJ/mol at  $-10$  °C versus  $-10.5$  kJ/mol at  $30$  °C (see Supporting Information for calculation). For higher catalyst loadings, the effect of temperature on the conversion is smaller. This may indicate that at certain catalyst loadings the polymerization is under kinetic rather than thermodynamic control due to the short residence times and rapid quenching of catalyst.

Next we investigated whether this is a living polymerization under our conditions. At 0.25 mol % catalyst loading and a reaction temperature of  $-10$  °C, the plot of  $M_n$  vs conversion follows a straight line (see Figure 2, bottom). The conversion at  $-10$  °C increased with increasing residence time, whereas the conversion at  $10$  °C leveled off after 3 s residence time (see Figure 2, top). At  $30$  °C, however, the conversion even decreased slightly at longer residence times. This clearly disagrees with the characteristics of a living polymerization. This lower conversion at longer residence times may be due to the competing depolymerization reaction that accelerates at higher temperatures, caused by the thermodynamic equilibrium between monomer and polymer. The depolymerization of PLA by transesterification is also catalyzed by TBD (see Figure 1) and can under certain circumstances compete with chain growth.

We investigated this further for both low and high catalyst loadings. At a catalyst loading of 0.1 mol % and a reaction temperature of  $-10$  °C, the polymerization conditions were systematically varied for that loading and a targeted DP of 80. No polymeric product was, however, observed at 2–5 s residence time by direct precipitation of the microreactor output into diethyl ether. Increasing the residence time up to 30 s at a reaction temperature of  $-10$  °C only gave oligomeric species (DP 8–12 as measured by  $^1H$  NMR). For longer residence times, low flow rates are needed, and in the case of the  $1 \mu L$  internal volume microreactor this may lead to pulsating flows due to limitations of the syringe pumps. The conversions for several longer residence times (up to 30 s) were therefore determined using a microreactor with a larger internal volume and otherwise identical characteristics (see Figure S14)

and showed that the conversion *decreased* with increasing residence time. We reason that there is not enough catalyst to properly start the polymerization, while depolymerization negatively impacts polymer length at longer residence times.

At high catalyst loading (i.e., >0.5 mol % for DP = 80) the polymerization seems to be under kinetic control due to the timely addition of benzoic acid to quench TBD. The monomer equilibrium concentration is expected to be higher at higher temperatures; thus, the polymerization was performed with 0.6 mol % TBD at  $30$  °C. For these conditions, the conversion was  $\approx 98\%$  for residence times up to 20 s, while longer residence times (i.e., 30 s or more) caused the conversion to drop again with a concomitant rise in PDI (see Figure S24). We attribute this to the products of the polymerization being under kinetic control with residence times <20 s, while the thermodynamic equilibrium is attained at longer residence times.

These and previously described data suggest that there is both a distinct low and high concentration regime for the ROP of L-lactide with respect to TBD-catalyst loading. At low catalyst loading (less than 0.2 equiv of catalyst per initiator or 0.25 mol % catalyst for a targeted DP of 80), the reaction appears to be thermodynamically controlled; the plot of conversion versus residence time is as shown in Figure 2 (top). At high catalyst loading (more than 0.4 equiv of catalyst per initiator, 0.5 mol % catalyst for a targeted DP of 80) and low residence time (10 s or less), the reaction appears to be under kinetic control, the same plot now resembling Figure S8, and the timely addition of a quenching agent stops the reaction from reaching thermodynamic equilibrium.

It should be emphasized that all the trends and optimal PLA polymerization conditions, described in the previous sections, could only be identified very rapidly thanks to the possibility of fast reaction condition screening in a microreactor.

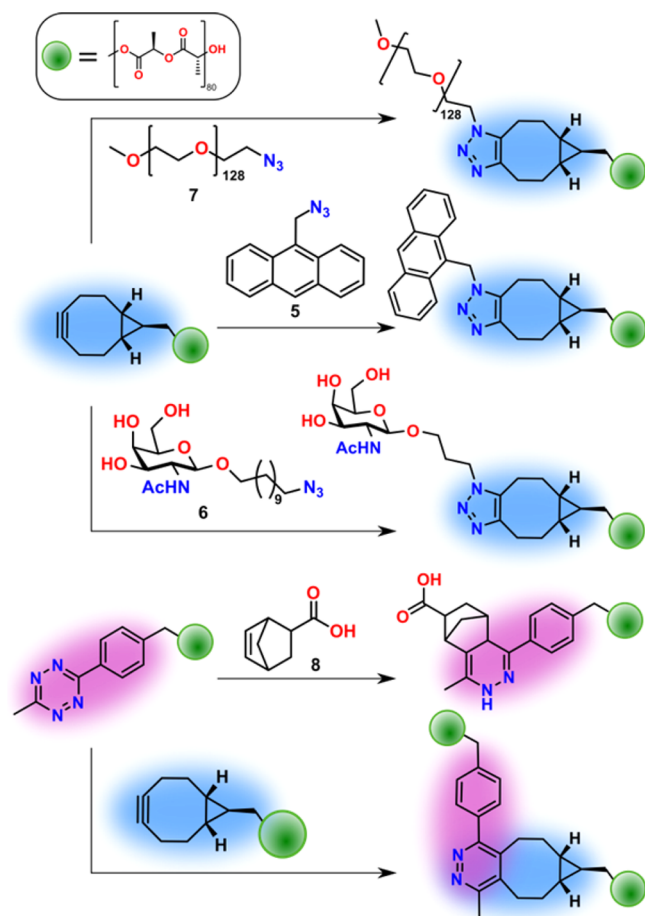
**Functionalized PLA Production in Flow Using Macro- and Click Chemistry-Ready Initiators.** Having demonstrated that the ring-opening polymerization of L-lactide can be performed outside of the glovebox in flow while maintaining good control over the process, we sought to add functionality to the resulting PLA polymer to increase the scope of its use in biomedical applications. We used our method for the preparation of PEG–PLA block copolymers, which are of interest for use in “stealthy” biocompatible drug carriers.<sup>32,33</sup> Using commercially available poly(ethylene glycol) monomethyl ether 2 ( $M_n$  5000 g/mol, PDI 1.04) as the initiator, the microreactor was used to quickly scan reaction conditions and generate PEG–PLA block copolymers in continuous flow.

At a catalyst loading of 1 mol %, a residence time of 2 s, and  $30$  °C, 68% conversion of monomer was observed, which increased to 96% conversion at a reaction temperature of  $-10$  °C. The resulting PEG–PLA block copolymers had a PDI of 1.13 and 1.23, respectively (see Figure S15).  $^1H$  NMR revealed that the composition of these two block copolymers was PEG<sub>113</sub>–PLA<sub>54</sub> and PEG<sub>113</sub>–PLA<sub>70</sub> (see Figures S3 and S4, respectively). Quantitative monomer conversion could be achieved using a 4 s residence time at  $-10$  °C, however, at the expense of a slightly higher PDI (1.32; DP of PLA block = 73).

Having demonstrated block copolymer synthesis initiated from macroinitiators, we sought to attach further functionality to the polymer end group. Strain-release-assisted click chemistries are, of course, a very versatile method to further functionalize PLA as they would allow for the facile and mild attachment of complex (bio)molecules without the involve-

ment of any metals. We identified the metal-free strain-promoted azide–alkyne and tetrazine–norbornene click reactions as fitting complements to our flow-based metal-free synthesis of PLA. As such, *exo*-BCN-derived alcohol **3** and tetrazine-linked alcohol **4** were investigated as potential initiators and click handles (see Figure 1) at a targeted DP of 80 with 1 mol % catalyst loading.

After screening reaction conditions, the polymerization starting from *exo*-BCN-derived alcohol **3** was performed at a reaction temperature of  $-10\text{ }^{\circ}\text{C}$  and 2 s residence time for 50 min. This yielded 150 mg of a white powder after centrifugation and drying (87%,  $M_n$  17 500 g/mol and PDI 1.24). In order to assess if the strained alkyne survived the polymerization process, a SPAAC reaction was performed using 9-(azidomethyl)anthracene (**5**) for 6 h (see Figure 6). After



**Figure 6.** SPAAC with PEG-5,000-N<sub>3</sub> (**7**), anthracene azide (**5**), and *N*-acetylgalactosamine derivative **6**, IEDDA with 5-norbornene-2-carboxylic acid (**8**), and finally IEDDA between BNC–PLA and tetrazine–PLA. The BCN moiety is highlighted in blue, and the tetrazine moiety is highlighted in purple.

purification,  $^1\text{H}$  NMR showed incorporation of the anthracene unit into the PLA, in a ratio of 1:164 (determined from the ratio of H-10 of the anthracene vs the backbone C–H protons of the PLA, matching the expected DP of 80), confirming the intact strained alkyne after polymerization (see Figure S2). To demonstrate the feasibility of attaching biomolecules, we carried out the SPAAC reaction between *N*-acetylgalactosamine derivative **6** and BCN–PLA (see Figure 6). After 20 h in hexafluoroisopropanol, the clicked glycopolymer was purified

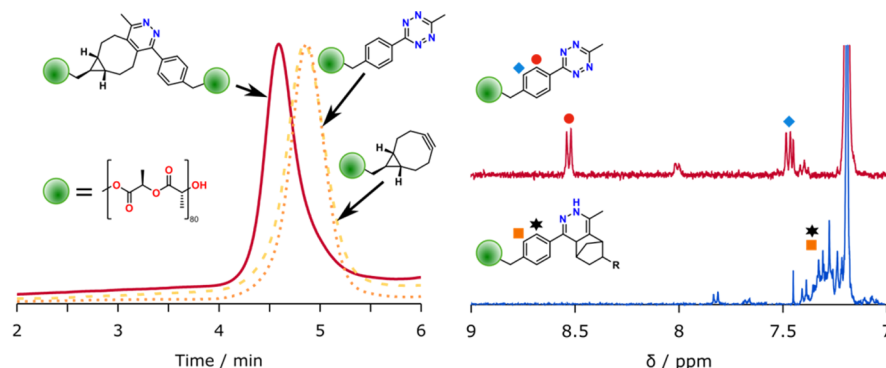
by filtration and precipitation, and  $^1\text{H}$  NMR confirmed full conversion of the BCN moiety (see Figure S6).

With our BCN–PLA polymer, also other polymeric materials containing an azide can be ligated, generating block copolymers. As an example, the SPAAC-ready BCN–PLA material was reacted with PEG-5000-N<sub>3</sub> (**7**) for 24 h at room temperature in DCM. Analysis by GPC showed full conversion of the BCN–PLA starting material to the targeted block copolymer (see Figure S16).

Analogously, we investigated tetrazine-linked alcohol **4** as an initiator for the ring-opening polymerization of *L*-lactide. Tetrazines are next-generation click chemistry handles and are amenable to the inverse electron demand Diels–Alder (IEDDA) reaction, which is among the fastest metal-free click reactions known.<sup>48</sup> The use of tetrazines in synthesis is currently limited by their incompatibility with highly basic catalysts such as DBU and TBD that cause them to degrade rapidly.<sup>40</sup> Methyl-substituted tetrazines such as **4** have been shown to be more stable than their hydrogen analogues. Nevertheless, we found that exposure of **4** to TBD in batch reactions caused rapid degradation.<sup>49</sup> On the basis of the short residence times needed for the polymerization of *L*-lactide in flow, however, we postulated that the very brief contact time between tetrazine **4** and TBD would limit tetrazine degradation in our flow setup. The tetrazine-derived alcohol **4** was added to the monomer solution and used as an initiator, with the TBD catalyst in a separate syringe. This led to brightly colored polymeric material as the output of the microreactor. At a catalyst loading of 1 mol %, high conversions of monomer were found, yielding a DP of 80 while keeping the PDI below 1.3. The polymerization was scaled out under these optimized conditions for 50 min (reaction temperature  $-10\text{ }^{\circ}\text{C}$ , residence time 2 s), giving 170 mg of a purple solid (98%,  $M_n$  17 000 g/mol, PDI 1.20) after purification by precipitation.  $^1\text{H}$  NMR analysis revealed a degree of polymerization of 80 units and also confirmed the intact nature of the tetrazine moiety in the polymer.

With this result we present the first example of tetrazine-terminated PLA and show a distinct advantage of continuous flow microreactors over batch chemistry, as the short reaction time enables the use of the base-labile tetrazine initiator **4** without causing degradation. Next, a small amount of purified tetrazine–PLA material was reacted for 16 h with 5-norbornene-2-carboxylic acid (**8**). The color of the solution changed from pink to colorless, and analysis by  $^1\text{H}$  NMR confirmed the complete consumption of the tetrazine handle (see Figure 7, right). A comparative study showed that methyl-substituted 1,2,4,5-tetrazines react slower than their hydrogen counterparts, which is as expected for an inverse electron demand Diels–Alder reaction.<sup>49</sup> Future development and use of different tetrazines may lead to faster reaction kinetics.

Finally, the efficiency of the inverse electron demand Diels–Alder reaction and use of both clickable PLAs were investigated by reacting BCN–PLA ( $M_n$  17 000 g/mol, DP 80) and tetrazine–PLA ( $M_n$  17 000 g/mol, DP 80) to generate larger PLA. After 16 h at room temperature, analysis by  $^1\text{H}$  NMR showed full consumption of BCN–PLA (see Figure S7). GPC shows a material with  $M_n$  33 000 g/mol and PDI 1.28 (see Figure 7, left). Facile precipitation yielded 70 mg of clicked polymeric product.



**Figure 7.** Left: GPC traces of BCN-PLA (dashed yellow) and tetrazine-PLA (dotted orange), both having DP = 80. The resulting clicked material (solid red) has  $M_n$  27 000 g/mol and PDI 1.28. Right: zoom of  $^1\text{H}$  NMR of tetrazine-PLA before (top) and after (bottom) inverse electron demand Diels-Alder reaction with 5-norbornene-2-carboxylic acid.

## CONCLUSION

The organocatalyzed ring-opening polymerization of L-lactide under continuous flow conditions produces well-defined PLA (PDI  $\approx$  1.2) within seconds at low catalyst loadings (0.25–1.2%) and high conversions (95–100%) without the use of an inert atmosphere during the polymerization. Use of the microreactor allows rapid extensive screening of reaction conditions for this polymerization. This revealed that longer residence times will typically give rise to higher conversions and a concomitantly broader molecular weight distribution due to transesterification of the polymer backbone by the catalyst (1,5,7-triazabicyclo[4.4.0]dec-5-ene; TBD). Polymers up to a DP of 240 ( $M_n$  44 000 g/mol) have been synthesized in the same continuous flow reactor by adjusting the initiator-to-monomer ratio. This organocatalytic, metal-free continuous flow method is rapid and mild enough to allow the use of an initiator that contains a PEG-5000 chain or a cyclooctyne or a base-labile tetrazine, without noticeable degradation of these moieties during the polymerization. The resulting BCN-PLA and tetrazine-PLA materials can then be readily functionalized with small (bio)molecules and large polymers bearing azides and norbornenes via SPAAC and inverse electron demand Diels-Alder click chemistry. Such functionalizations allow for an easy expansion of the scope of flow-based polymerizations for biomedical and other applications.

## MATERIALS AND METHODS

Tetrahydrofuran (THF, HiPerSolv CHROMANORM, VWR), 5-norbornene-2-carboxylic acid, **8** (98%, mixture of endo/exo, Sigma-Aldrich), methoxypoly(ethylene glycol) azide, **7** (Sigma-Aldrich, MW 5000 g/mol), and poly(ethylene glycol) monomethyl ether, **2** (Sigma-Aldrich, MW 5000 g/mol, PDI 1.04 as determined by GPC in THF vs PS standards), were used as received. Dichloromethane ( $\text{CH}_2\text{Cl}_2$ , Sigma-Aldrich, >99.8%) and toluene (Sigma-Aldrich, >99.8%) were purified over aluminum oxide under argon using a Pure Solv 400 solvent purification system (Innovative Technology, Amesbury, MA). (3S)-cis-3,6-Dimethyl-1,4-dioxane-2,5-dione (L-lactide, Aldrich, 98%) was recrystallized twice from dry toluene and dried under vacuum. Benzoic acid (Sigma-Aldrich, 99.5%) was used as received. 1,5,7-Triazabicyclo[4.4.0]dec-5-ene (TBD, Aldrich, 98%) is very moisture sensitive and was therefore stored in a glovebox under an argon atmosphere. 4-tert-Butylbenzyl alcohol, **1** (Aldrich, 98%), was stored on 4 Å mol sieves. ((1R,8S,9R)-Bicyclo[6.1.0]non-4-yn-9-yl)methanol (exo-BCN-alcohol **3**)<sup>35</sup> and 9-(azidomethyl)anthracene, **5**, were prepared according to literature procedures.<sup>50</sup> (4-(6-Methyl-1,2,4,5-tetrazin-3-yl)phenyl)methanol (tetrazine **4**) was prepared according to a literature procedure,<sup>51</sup> purified with dry column vacuum chromatography (heptane:ethyl acetate, 0%–60%, 5% increase in

ethyl acetate per fraction) and recrystallized from heptane:ethyl acetate (10:1). N-Acetylgalactosamine derivative **6** was prepared according to a modified literature procedure (see Supporting Information).<sup>52</sup>

**Characterization.**  $^1\text{H}$  NMR experiments were performed on a Bruker Avance III 400 MHz spectrometer. The degree of polymerization for PLA initiated from 4-tert-butylbenzyl alcohol was determined by integrating the backbone protons at 5.15 ppm and the tert-butyl protons of the initiator at 1.32 ppm. Conversion was determined by integrating the monomer signals at 5.05 ppm and the polymer signals at 5.15 ppm. Molecular weight distribution was characterized by gel-permeation chromatography (GPC), performed using an Agilent 1200 LC system fitted with a PLgel 10  $\mu\text{m}$  MIXED-D column. THF was used as a solvent and the system was calibrated using monodisperse polystyrene standards. MALDI-ToF-MS was performed using 2 mg/mL concentration using 2-[(2E)-3-(4-tert-butylphenyl)-2-methylprop-2-enylidene]malononitrile (DCTB)] as a matrix and KTFA (potassium trifluoroacetate) as a matrix additive. The measurement was performed on a Bruker Autoflex Speed instrument.

**Typical Polymerization of L-Lactide in Continuous Flow.** In a glovebox, 14.5 mg of TBD was weighed into a 25 mL volumetric flask. Outside of the glovebox, under a flow of nitrogen, 5 mL of a 0.1 M DCM solution of the initiator (4-tert-butylbenzyl alcohol **1**) was added. The resulting suspension was sonicated for 5 min. Dry DCM was added to the 25 mL mark, and the solution was sonicated again for 5 min. Recrystallized L-lactide (2.3 g) was weighed in a 10 mL volumetric flask. Dry DCM was added to the 10 mL mark to get a 1.6 M monomer solution. Finally, benzoic acid (122 mg) was dissolved in 5 mL of dry DCM. Three syringes were filled with initiator/catalyst, monomer, and quench solution and loaded onto syringe pumps. The Chemtrix Labtrix system was fitted with a SOR 3221 chip (1  $\mu\text{L}$  reactor volume, staggered oriented ridge mixers). The residence time was set by the flow rate of the syringe pumps; for a 2 s residence time, the first syringe pump (delivering the initiator/catalyst and monomer solutions) was set to 15  $\mu\text{L}/\text{min}$  and the second syringe pump (delivering the benzoic acid quench solution) was set to 30  $\mu\text{L}/\text{min}$ . Previous findings by Tonhauser et al. showed that flushing the reactor prior to the reaction may remove adventitious contaminants, such as water.<sup>2</sup> Water will quench TBD or act as an initiator, lowering the effective catalyst loading and/or leading to uncontrolled polymerization. Prior to collection of samples, the microreactor was flushed with the solutions for 10–20 min. After equilibration for 10 min at  $-10^\circ\text{C}$ , a sample was collected in a GC vial. After sample collection, the temperature was increased to  $30^\circ\text{C}$ , and again, the setup was equilibrated and a sample was taken. Finally, the temperature was decreased to  $10^\circ\text{C}$ , and a sample was taken after equilibration. This operation was repeated for other residence times, in random order. At each residence time, the three experiments at different temperatures were performed in random order.

**Batch Polymerization outside of Glovebox.** In the glovebox, 21.1 mg of TBD was weighed into a 10 mL volumetric flask. Outside

of the glovebox, 2 mL of a solution of 4-*tert*-butylbenzyl alcohol solution (0.1 M, 0.164 g in 10 mL of DCM) and DCM was added to the 10 mL mark. A fresh monomer solution (1.6 M, 1.15 g in 5 mL of DCM) was prepared. 0.2 mL of each solution was added to oven-dried vials under a nitrogen flow. The temperature in the lab was 20 °C. After 3 s, 0.5 mL of a benzoic acid solution (0.2 M, 0.23 g in 10 mL of DCM) was added to quench the reaction. The solvent was removed by nitrogen flow. The conversion was measured using <sup>1</sup>H NMR.

To compare these results with flow, the same solutions were used in the microreactor setup. The temperature was set to 20 °C, the same as in the lab. After an equilibration time of 20 min, the output was collected and analyzed by <sup>1</sup>H NMR after removal of solvent.

**L-Lactide Polymerization Initiated from BCN-Alcohol 3.** As above, TBD (32 mg, 0.23 mmol) was weighed into a 10 mL volumetric flask inside the glovebox. To this flask was added *exo*-BCN-alcohol 3 (28 mg, 0.19 mmol). The polymerization in the microreactor was performed as above. After obtaining optimal reaction conditions, the output of the microreactor was collected for 50 min to yield 152 mg BCN-PLA.

**L-Lactide Polymerization Initiated from Tetrazine-Alcohol 4.** As above, TBD (27.0 mg, 0.19 mmol) was weighed into a 10 mL volumetric flask inside the glovebox. Under the above conditions (TBD and initiator in one flask), the tetrazine is not stable. Therefore, a new stock solution of L-lactide was made (1.15 g, 7.99 mmol) in 5 mL of distilled DCM. To this flask was added tetrazine-derived alcohol 4 (20 mg, 0.10 mmol). The polymerization in the microreactor was performed as above. After obtaining optimal reaction conditions, the output of the microreactor was collected for 50 min to yield 171 mg of tetrazine-PLA.

**SPAAC Reaction with Azidoanthracene and BCN-PLA.** To 12 mg of BCN-PLA (0.001 mmol) in DCM (1 mL) was added 9-(azidomethyl)anthracene 5 (5.5 mg, 0.024 mmol). This solution was stirred at rt for 6 h. After this time, the polymer was precipitated twice in diethyl ether and isolated by centrifuge at 5000 rpm for 2 min. The resulting white precipitate was dried *in vacuo* and analyzed using GPC and <sup>1</sup>H NMR.

**SPAAC Reaction with PEG-5000-N<sub>3</sub> and BCN-PLA.** In a GC vial was 27 mg of PEG-5000-N<sub>3</sub> 2 (0.0054 mmol). To this was added 1.0 mL of solution of BCN-PLA in DCM (0.005 mmol, M<sub>n</sub> 28 000 g/mol, PDI 1.24), and the resulting solution was stirred at rt. After 24 and 48 h, samples were taken and analyzed using GPC.

**SPAAC Reaction with N-Acetylgalactosamine Derivative 6 and BCN-PLA.** To 12 mg of BCN-PLA (0.001 mmol) in hexafluoroisopropanol (0.5 mL) was added N-acetylgalactosamine derivative 6 (3.0 mg, 0.037 mmol). This solution was stirred at rt for 20 h. After this time, the suspension was filtered over cotton and precipitated into diethyl ether. After isolation by centrifugation (5000 rpm, 1 min), the resulting solid was redissolved in DCM and filtered again over cotton. The solvent was removed *in vacuo*, and the resulting polymer was analyzed using <sup>1</sup>H NMR.

**Inverse Electron Demand Diels–Alder Reaction with Tetrazine-PLA and 5-Norbornene-2-carboxylic Acid.** To 15 mg of purified tetrazine-PLA (0.0013 mmol) in 0.5 mL of DCM was added five drops of 5-norbornene-2-carboxylic acid (8). The resulting purple solution was kept at rt for 16 h. After this time, the solution had turned colorless. The polymer was precipitated twice from diethyl ether and analyzed by <sup>1</sup>H NMR.

**Inverse Electron Demand Diels–Alder Reaction with Tetrazine-PLA and BCN-PLA.** To 47 mg of tetrazine-PLA (0.004 mmol) was added 50 mg of BCN-PLA (0.004 mmol) and DCM (1 mL). The resulting purple solution was stirred at rt for 16 h, after which it was nearly colorless, purified by precipitation from diethyl ether, and analyzed with GPC and <sup>1</sup>H NMR.

## ■ ASSOCIATED CONTENT

### ■ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.macromol.5b02533.

<sup>1</sup>H NMR spectra of polymers and compounds, tabulated data of polymerization reactions, GPC traces, and selected graphs (PDF)

## ■ AUTHOR INFORMATION

### Corresponding Authors

\*E-mail [han.zuillhof@wur.nl](mailto:han.zuillhof@wur.nl) (H.Z.).

\*E-mail [t.wennekes@uu.nl](mailto:t.wennekes@uu.nl) (T.W.).

### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

This work is supported by NanoNextNL (program 10C), a micro and nanotechnology consortium of the Government of The Netherlands and 130 partners, and The Netherlands Organization for Scientific Research (NWO VENI grant 722.011.006). The authors thank Dr. Jaime Garcia Hartjes for synthesizing and characterizing GalNAc derivative 6, Judith Firet for synthesis of BCN-alcohol 3, and Dr. Maarten Smulders for insightful discussions. The authors thank Henk Janssen and Xianwen Lou (TU/e, Eindhoven) for MALDI-ToF-MS measurements.

## ■ REFERENCES

- (1) Wilms, D.; Klos, J.; Frey, H. *Macromol. Chem. Phys.* **2008**, *209*, 343–356.
- (2) Tonhauser, C.; Wilms, D.; Wurm, F.; Nicoletti, E. B.; Maskos, M.; Löwe, H.; Frey, H. *Macromolecules* **2010**, *43*, 5582–5588.
- (3) Iida, K.; Chastek, T. Q.; Beers, K. L.; Cavicchi, K. A.; Chun, J.; Fasolka, M. J. *Lab Chip* **2009**, *9*, 339–345.
- (4) Baeten, E.; Verbraken, B.; Hoogenboom, R.; Junkers, T. *Chem. Commun. (Cambridge, U. K.)* **2015**, *51*, 11701–4.
- (5) Geyer, K.; Codee, J. D.; Seeberger, P. H. *Chem. - Eur. J.* **2006**, *12*, 8434–8442.
- (6) Tonhauser, C.; Natalello, A.; Löwe, H.; Frey, H. *Macromolecules* **2012**, *45*, 9551–9570.
- (7) Simón, L.; Goodman, J. M. *J. Org. Chem.* **2007**, *72*, 9656–9662.
- (8) Kiesewetter, M. K.; Scholten, M. D.; Kim, N.; Weber, R. L.; Hedrick, J. L.; Waymouth, R. M. *J. Org. Chem.* **2009**, *74*, 9490–9496.
- (9) Hornung, C. H.; Guerrero-Sanchez, C.; Brasholz, M.; Saubern, S.; Chiefari, J.; Moad, G.; Rizzardo, E.; Thang, S. H. *Org. Process Res. Dev.* **2011**, *15*, 593–601.
- (10) Derboven, P.; Van Steenberge, P. H. M.; Vandenberghe, J.; Reyniers, M.-F.; Junkers, T.; D'Hooge, D. R.; Marin, G. B. *Macromol. Rapid Commun.* **2015**, *36*, 2149–2155.
- (11) Shen, Y.; Zhu, S. *AIChE J.* **2002**, *48*, 2609–2619.
- (12) Diehl, C.; Laurino, P.; Azzouz, N.; Seeberger, P. H. *Macromolecules* **2010**, *43*, 10311–10314.
- (13) Kundu, S.; Bhangale, A. S.; Wallace, W. E.; Flynn, K. M.; Guttman, C. M.; Gross, R. A.; Beers, K. L. *J. Am. Chem. Soc.* **2011**, *133*, 6006–11.
- (14) Natalello, A.; Morsbach, J.; Friedel, A.; Alkan, A.; Tonhauser, C.; Müller, A. H. E.; Frey, H. *Org. Process Res. Dev.* **2014**, *18*, 1408–1412.
- (15) Melker, A.; Fors, B. P.; Hawker, C. J.; Poelma, J. E. *J. Polym. Sci., Part A: Polym. Chem.* **2015**, *53*, 2693–2698.
- (16) Leibfarth, F. A.; Johnson, J. A.; Jamison, T. F. *Proc. Natl. Acad. Sci. U. S. A.* **2015**, *112*, 10617–10622.
- (17) Jamshidian, M.; Tehrani, E. A.; Imran, M.; Jacquot, M.; Desobry, S. *Compr. Rev. Food Sci. Food Saf.* **2010**, *9*, 552–571.
- (18) Institute for Bioplastics and Biocomposites: Material share of biopolymer production capacity. <http://ifbb.wp.hs-hannover.de/downloads/content/Statistics/Market%20statistics/Production%20capacities/By%20material%20type/2013/Material%20share%20of%20biopolymer%20production%20capacity%20sorted%20by%20material%20grade%202013.png> (August 25, 2015).

- (19) Dove, A. P. *ACS Macro Lett.* **2012**, *1*, 1409–1412.
- (20) Lohmeijer, B. G. G.; Pratt, R. C.; Leibfarth, F.; Logan, J. W.; Long, D. A.; Dove, A. P.; Niederberg, F.; Choi, J.; Wade, C.; Waymouth, R. M.; Hedrick, J. L. *Macromolecules* **2006**, *39*, 8574–8583.
- (21) Saito, T.; Aizawa, Y.; Tajima, K.; Isono, T.; Satoh, T. *Polym. Chem.* **2015**, *6*, 4374–4384.
- (22) Dove, A. P.; Li, H.; Pratt, R. C.; Lohmeijer, B. G. G.; Culkin, D. A.; Waymouth, R. M.; Hedrick, J. L. *Chem. Commun.* **2006**, 2881–2883.
- (23) Guillermin, B.; Lemaire, V.; Ernould, B.; Cornil, J.; Lazzaroni, R.; Gohy, J.-F.; Dubois, P.; Coulembier, O. *RSC Adv.* **2014**, *4*, 10028.
- (24) Jung, H.; Brummelhuis, N. t.; Yang, S. K.; Weck, M. *Polym. Chem.* **2013**, *4*, 2837.
- (25) Barker, I. A.; Hall, D. J.; Hansell, C. F.; Du Prez, F. E.; O'Reilly, R. K.; Dove, A. P. *Macromol. Rapid Commun.* **2011**, *32*, 1362–6.
- (26) Pratt, R. C.; Lohmeijer, B. G. G.; Long, D. A.; Waymouth, R. M.; Hedrick, J. L. *J. Am. Chem. Soc.* **2006**, *128*, 4556–4557.
- (27) Horn, H. W.; Jones, G. O.; Wei, D. S.; Fukushima, K.; Lecuyer, J. M.; Coady, D. J.; Hedrick, J. L.; Rice, J. E. *J. Phys. Chem. A* **2012**, *116*, 12389–12398.
- (28) Leibfarth, F. A.; Moreno, N.; Hawker, A. P.; Shand, J. D. *J. Polym. Sci., Part A: Polym. Chem.* **2012**, *50*, 4814–4822.
- (29) Garcia Hartjes, J.; Bernardi, S.; Weijers, C. A. G. M.; Wennekes, T.; Gilbert, M.; Sansone, F.; Casnati, A.; Zuillhof, H. *Org. Biomol. Chem.* **2013**, *11*, 4340–4349.
- (30) Kallemeyn, W. W.; Witte, M. D.; Wennekes, T.; Aerts, J. M. F. G.; Derek, H. In *Advances in Carbohydrate Chemistry and Biochemistry*; Academic Press: 2014; Vol. 71, Chapter 4, pp 297–338.
- (31) Duval, F.; van Beek, T. A.; Zuillhof, H. *Analyst* **2015**, *140*, 6467–6472.
- (32) Verrecchia, T.; Spenlehauer, G.; Bazile, D. V.; Murry-Brelier, A.; Archimbaud, Y.; Veillard, M. *J. Controlled Release* **1995**, *36*, 49–61.
- (33) Mackiewicz, N.; Nicolas, J.; Handké, N.; Noiray, M.; Mougin, J.; Daveu, C.; Lakkireddy, H. R.; Bazile, D.; Couvreur, P. *Chem. Mater.* **2014**, *26*, 1834–1847.
- (34) Lutz, J. F. *Angew. Chem., Int. Ed.* **2007**, *46*, 1018–25.
- (35) Dommerholt, J.; Schmidt, S.; Temming, R.; Hendriks, L. J.; Rutjes, F. P.; van Hest, J. C.; Lefebvre, D. J.; Friedl, P.; van Delft, F. L. *Angew. Chem., Int. Ed.* **2010**, *49*, 9422–5.
- (36) Guo, J.; Chen, G.; Ning, X.; Wolfert, M. A.; Li, X.; Xu, B.; Boons, G. J. *Chem. - Eur. J.* **2010**, *16*, 13360–13366.
- (37) Zheng, J.; Liu, K.; Reneker, D. H.; Becker, M. L. *J. Am. Chem. Soc.* **2012**, *134*, 17274–7.
- (38) Ledin, P. A.; Kolishetti, N.; Hudlikar, M. S.; Boons, G. J. *Chem. - Eur. J.* **2014**, *20*, 8753–8760.
- (39) Wang, S.; Yang, X.; Zhu, W.; Zou, L.; Zhang, K.; Chen, Y.; Xi, F. *Polymer* **2014**, *55*, 4812–4819.
- (40) Hansell, C. F.; Espeel, P.; Stamenovic, M. M.; Barker, I. A.; Dove, A. P.; Du Prez, F. E.; O'Reilly, R. K. *J. Am. Chem. Soc.* **2011**, *133*, 13828–31.
- (41) A residence time of 2 s is almost the fastest that this setup allows. Shorter residence times require higher flow rates that may damage the microreactor chip.
- (42) Duda, A.; Kowalski, A.; Libiszowski, J.; Penczek, S. *Macromol. Symp.* **2005**, *224*, 71–84.
- (43) Witzke, D. R.; Narayan, R.; Kolstad, J. J. *Macromolecules* **1997**, *30*, 7075–7085.
- (44) Micic, N.; Young, A.; Rosselgong, J.; Hornung, C. *Processes* **2014**, *2*, 58–70.
- (45) Martello, M. T.; Burns, A.; Hillmyer, M. *ACS Macro Lett.* **2012**, *1*, 131–135.
- (46) Tuba, R.; Grubbs, R. H. *Polym. Chem.* **2013**, *4*, 3959–3962.
- (47) Hong, M.; Chen, E. Y. X. *Nat. Chem.* **2016**, *8*, 42–49.
- (48) Knall, A.-C.; Slugovc, C. *Chem. Soc. Rev.* **2013**, *42*, 5131–42.
- (49) Karver, M. R.; Weissleder, R.; Hilderbrand, S. A. *Bioconjugate Chem.* **2011**, *22*, 2263–2270.
- (50) Tosic, O.; Mattay, J. *Eur. J. Org. Chem.* **2011**, 2011, 371–376.
- (51) Yang, J.; Karver, M. R.; Li, W.; Sahu, S.; Devaraj, N. K. *Angew. Chem., Int. Ed.* **2012**, *51*, 5222–5.
- (52) Mandal, S.; Sharma, N.; Mukhopadhyay, B. *Synlett* **2009**, 2009, 3111–3114.