Progress Toward Reaction Monitoring at Variable Temperatures: A New Stopped-Flow NMR Probe Design

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Abstract

A stopped-flow NMR probe is described that enables fast flow rates, short transfer times, and equilibration of the reactant magnetization and temperature prior to reaction. The capabilities of the probe are demonstrated by monitoring the polymerization of lactide as catalyzed by the air-sensitive catalyst 1,3-dimesitylimidazol-2-ylidene (IMes) over the temperature range of -30 to 40 °C. The incorporation of stopped-flow capabilities into an NMR probe permits the rich information content of NMR to be accessed during the first few seconds of a fast reaction.

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Introduction

Nuclear magnetic resonance (NMR) spectroscopy provides detailed kinetic and mechanistic information and, hence, is one of the most valuable techniques for monitoring chemical reactions in solution.[1] However, the most common technique for following reactions by NMR spectroscopy, by which reagents are injected into an NMR tube then placed in the spectrometer for measurement, is blind to the first *ca.* 30 s of reaction. Therefore, other methods for introduction of the sample into the NMR instrument are needed to observe faster time scales.

Common methods for accessing fast reactions include variable temperature (VT) NMR, rapid injection NMR, and stopped-flow NMR. The VT approach to following fast reactions by NMR is to slow the reaction by lowering the temperature. However, VT NMR limits the choice of solvent and may move the reaction conditions outside the region of interest. (We note that NMR exchange spectroscopy in combination with VT measurements provides detailed kinetic information over a broad temperature range only for reversible reactions.[2–6])

Several rapid injection methods allow kinetic elucidation for irreversible reactions with half-lives in the sub-second regime.[7–17] Rapid injection techniques include methods that effect mixing through mechanical stirrers or through the impact of the jet of fast moving reagent solution. In the latter case it appears that mixing times can be as short as 30 ms. Advantages of the rapid injection technique include compatibility with standard NMR probes and standard temperature control systems. However, the rapid injection technique is a batch method which makes high throughput data collection more difficult than flow systems.

Stopped-flow (SF) NMR spectrometry enables rapid measurement of fast reactions with the convenience of a flow system.[18–25] SF NMR involves pushing two solutions (here displayed as reagents in separate driving syringes) rapidly through an efficient mixer and then the detection region of the NMR instrument (Figure 1). After the active region has been completely flushed by the flowing solution, the flow is rapidly stopped and data acquisition is initiated. Figure 1 displays a common stopping method where spent reaction mixture collects in a stopping syringe which then hits a physical barrier (or stop plate). The flow must be stopped prior to acquisition due to the high flow rates (*ca*. 10 mL/s) leading to inadequate residence time in the detection region.

An important consideration is the amount of time which it takes the solutions to mix and reach the middle of the active region, known as the transport time. In addition, the solutions must stop flowing prior to data collection; this time is defined as the stop time. The dead time, the shortest time necessary to wait before a spectrum is acquired, is the sum of stop and transport times. A related measure is the mixing time, which represents the time need to achieve complete mixing of the two reactant solutions.

Applications of SF NMR in the literature are wide-spread and include biological, organic and inorganic reactions.[18,20–23,25–31] Dead times as low as a few milliseconds have been reported, but mixing efficiency, the extent of sample magnetization, and ability to operate with variable temperatures are not always addressed. Previously our group described a simple stopped-flow NMR apparatus that incorporates a mixer assembly into a flow NMR probe and a pneumatic drive system.[28] A disadvantage of this design was the lack of temperature control. In this paper we describe a custom modification of an NMR probe that enables improved reagent magnetization, high flow rates, fast mixing, and variable temperature capabilities.

Stopped-Flow NMR Probe Design

The stopped-flow NMR system comprises a solution drive system, an NMR probe that was modified to accommodate the mixer and reaction zone, and an electronic signal system that detects stoppage of flow and communicates to the NMR console through a transistor–transistor logic (TTL) circuit. The solution drive system, which is mounted to a plastic cart that is placed close to the NMR magnet, has been described by us in a previous publication.[28] For the data reported herein, the originally described drive system was modified slightly by minimizing turns in the flow path and maintaining more constant cross sectional areas throughout the flow lines (see Supporting Information).

The SF NMR probe is a modified wide-bore Bruker 360 MHz NMR probe. Overall the flow path consists of the reactant lines that enter the probe body through the bottom and emerge at the probe top, a rapid mixing cell, and a single glass flowtube reactor that begins at the mixer, passes through the NMR detection coils, and continues down through the bottom of the probe body.

The stopped flow mixer is a T-shaped, two jet (two reagent streams) mixer that allow for high flow rates and low transport times. The T-shape flow path generates high turbulence at the junction that facilitates rapid mixing of the reagents prior to reaching the detection region. The mixer is composed of three main parts: the cap, body, and base (Figure 2) that are custom machined out of polychlorotrifluoroethylene (PCTFE).

The reagents flow through holes **A** and **D** once the cap is secured to the body with four PCTFE screws. Ethylene tetrafluoroethylene polymer (ETFE) reagent lines (1/8 inch outer diameter (OD), 0.062 inch inner diameter (ID)) screw into the body of the mixer with polyether ether ketone (PEEK) flangeless fitings and ETFE ferrules at **C**. A seal between the glass flowtube and the mixer is achieved with an o-ring compressed by the cap. Grooves in the body serve as guides to allow the reagent lines to coil in an alternating fashion about the

detection region. The acquisition coil (3 mm) is located about the flowtube in the center of the hollow cylinder body. Copper foil is wrapped around the inside of the hollow cylinder to provide shielding of the reagents from the detection coil. The hollow cylinder is press fit into the PCTFE base.

The coiling of the reagent flow lines around the outside of the mixer assembly was incorporated to facilitate thermal and magnetic equilibration of the reactants prior to mixing. When a stopped-flow "shot" is executed, the volume of reagents that mix and enter the NMR detection region are wholly contained within the coiled delivery lines. Because these lines lie in a temperature controlled region at the center of the magnetic field, both the temperature and magnetization of the reactants are equilibrated prior to reaction initiation.

The flowtube consists of a 6 cm length of 3 mm OD NMR tube within the detection region which is fused with 3 mm glass tubing to extend through the entire NMR probe. At the bottom of the NMR probe a Cajon-type o-ring fitting secures the flowtube to the NMR probe. The flowtube is connected with high-performance liquid chromatography (HPLC) fittings to ETFE tubing which is connected to the pull side of the push-pull pneumatic driver.

Temperature control is achieved by using the standard Bruker gas purge system equipped with a heating coil; for sub-ambient temperatures the gas first circulates through coils submerged in a low temperature bath or Dewar and the gas temperature is controlled by the subsequent heating coil. Temperature control of the reagent lines, mixer assembly, and reactor zone is facilitated by capping the top of the probe with a glass Dewar (see Further Details). A thermocouple is routed from the bottom of the probe to the base of the reactor zone to measure the temperature within the Dewar cap. It is connected to a standard Bruker thermocouple connection to allow the spectrometer to monitor the temperature inside the Dewar cap for calibration.

Results and Discussion

In order to demonstrate the capabilities of our stopped-flow NMR probe, we elected to study a reaction which has a rate that is mostly independent to temperature, the polymerization of lactide by the organocatalyst 1,3-dimesitylimidazol-2-ylidene (IMes). The polymerization of lactide by IMes has been well studied in the literature (Scheme 1).[32,33] The N-heterocyclic carbene (NHC) catalyst IMes quickly deactivates upon exposure to moisture, so successful polymerizations demonstrate that this SF NMR system is moisture-free and presumably air-free. As the system and reagent lines are used and stored outside of a dry box, the reagent lines are first dried with a toluene solution of $Al(^{i}Bu)_2(BHT)$ (BHT = 2,6 di-tert-butyl-4-methylphenol) prior to introducing monomer and catalyst solutions.

For detailed mechanistic or kinetic studies, it is necessary to collect NMR spectra with a high signal-to-noise (S/N) ratio to allow for accuracy of integrations. To provide evidence that this probe is capable of such work, reagent solutions were prepared in toluene-d_8 (a total of 10 mL toluene-d_8) to reduce the toluene signal and allow for better S/N. The ^{1}H NMR spectra of reaction progression are displayed in Figure 3. The monomer used was 89% *meso*-lactide and 11% *rac*-lactide, and equilibration to the more thermodynamically favorable *rac*-lactide *via* epimerization is observed over the course of the first few spectra. Observation of the *rac-meso* equilibration is not possible using traditional NMR techniques because equilibration is complete within *ca.* 10 s. Growth of polymer with time was clearly observed, demonstrating the fast time resolution and inert conditions that can be achieved with this probe. Addition of known amounts of internal standard 1,4-bis(trimethylsilyl)benzene (BTMSB) allowed for the determination of the absolute concentration of each species in solution. However, it should be noted that these concentrations are based upon no dilution of each reagent solution. There is likely dilution of the reagent solutions with the solvent due to mixing upon injection of reagent solutions.

Additionally, lactide polymerizations were run in protio toluene to demonstrate more relevant, cost-effective reaction monitoring conditions, albeit with lower S/N resulting from the use of protio solvent. Figure 4 displays the reaction progression at 0 °C in protio toluene.

These data were fit to a simple kinetic model to gain understanding of the epimerization and polymerization rate constants at 0 °C in protio toluene (Scheme 2). The fit of the model (Figure 5) is good even with the noisy raw data. The model converged to k_{mr} = 0.024 ± 0.001 s^{-1} and k_{prop} = 0.0084 ± 0.0003 $M^{-1}s^{-1}$. The rate constant k_{rm} converged to a value of zero, indicating that once *meso*-lactide epimerizes to the more thermodynamically stable *rac*-lactide it is quickly incorporated into the polymer without further measurable epimerization. All errors reported are the standard deviations. Even in protio solvent with low S/N, valuable kinetic information is obtained by fitting the data to a kinetic model.

To further demonstrate the utility of the stopped-flow NMR probe, lactide polymerization was performed at -30, -10, 0, 25 and 40 °C in protio toluene (see Further Details). There is not a strong temperature dependence for the rate of polymerization, indicative of a low apparent activation enthalpy for the catalytic reaction. The ability to successfully monitor lactide polymerization at a variety of temperatures indicates that the SF probe is suitable to study a wide variety of reactions. Deutero solvent is not necessary for reaction monitoring, allowing cost-effective studies.

Conclusion

Stopped-flow NMR enables study of fast reactions using the high intrinsic information content of NMR spectroscopy. We designed a new stopped-flow NMR probe by modifying an existing wide-bore probe utilizing a coiled tube reservoir located near the center of the magnet that enables reagent magnetization and temperature equilibration prior to mixing. The probe exhibits a fast flow rate (14.5 mL/s) and short transport time (2.6 ms) that

assure rapid and complete mixing and short dead times for fast reactions. However, the signal quality as revealed by NMR linewidths is deteriorated for the first *ca.* 100 ms after the stopped-flow trigger activates. This signal deterioration may reflect some residual flow through the active region and/or vibrations of the detection coil and reactor tube. The difference in temperature between the coiled tube reservoir and the NMR-detected active region is less than 4 °C. The polymerization of lactide with the air-sensitive catalyst IMes were performed over the temperature range of -30 to 40 °C. This SF NMR probe is especially valuable for monitoring fast reactions where traditional NMR techniques would suffer from incomplete mixing, poor temperature control, and missing the first *ca*. 30 s of reaction.

Further Details

Temperature Control and Regulation

Temperature control of reagent lines, mixer, and active region is achieved by placing a glass Dewar around the entire assembly (Figure 6). The standard Bruker gas purge system and heating coil are utilized to change the temperature.

To test the variable temperature capabilities of the probe, a temperature calibration was computed using the difference of the chemical shifts of neat methanol (Figure 7).[34] Spectra were successfully measured at a temperature range of -43 to 46 °C with no observable problems.

The temperatures of the reagent lines within the Dewared cap and the active region within the mixer body, which is not directly exposed to the cooling gas, could be different. If the reagent lines and active region are at different temperatures, there will be a temperature gradient upon solvent push. In order to measure the temperature change upon solvent push into the detection region, the system was filled with neat methanol and set to 0 °C. Methanol

(0.2 mL per line, 0.4 mL total) was pushed through the system and a series of spectra was acquired to allow measurement of the temperature variation upon solvent push and time to temperature equilibrium (Figure 8).

As displayed in Figure 8, the temperature of methanol detected in the active region of the NMR decreases by about 3.5 °C when 0.4 mL of solvent is pushed from the equilibration region of the reagent lines into the active region. This indicates that the equilibration region is slightly colder than the set point of the active region. The time for temperature equilibration to the set 0 °C is observed to be approximately 300 seconds. The small (< 4 °C) temperature differential between the equilibration and detection regions should not cause a problem for most kinetic measurements.

Mixing Ability of the Stopped-Flow Probe

The mixing capability of a T-shaped mixer design was tested with a prototype (Figure 9). A basic indicator solution of bromothymol blue (left) was mixed with an acidic solution (right) at a flow rate of ~8-10 mL/s. The rapid acid-base reaction results in a color change, so any blue color in the center region would be indicative of incomplete mixing. The photo shows no blue solution after leaving the mixer, indicating that the T-shape mixing design results in complete mixing of the two solutions.

Another test of mixing capability is to observe ^{1}H NMR spectra resulting from mixing acetone and chloroform. The chemical shift of neat chloroform is different by almost 1 ppm from the chemical shift of chloroform in a homogenous solution with acetone. Incomplete mixing of chloroform and acetone results in distinctive broadening of the peaks (Figure 10).

Thus, passing chloroform and acetone solutions through the mixer in a stopped-flow experiment enables the mixing efficiency to be gauged from the line width of the chloroform signal. The mixing test was first performed by filling the entire system with acetone, then injecting chloroform into one of the reagent lines. Upon mixing, one signal for chloroform was observed which slightly shifts upfield with time (Figure 11). However, it is likely that the chloroform mixed with the acetone that was previously in the reagent line. The integrations of the two signals display that there is much more acetone in solution than chloroform which supports the premixing hypothesis. If the chloroform did not mix with the acetone in the reagent lines, the spectra would display a 1:1 mixture of chloroform:acetone. Therefore, this approach is not a conclusive test of mixing ability.

The next approach to perform the chloroform and acetone mixing test was to fill the system with a third solvent and inject equal amounts of chloroform and acetone in order to obtain a 1:1 mixture. The system was filled with toluene and chloroform and acetone were injected into separate reagent lines. Upon mixing, one signal for chloroform was observed (Figure 12), but there is a significant amount of toluene in solution. The presence of toluene will change the chemical shift values of chloroform and acetone, making it difficult to interpret chemical shift changes upon mixing. Although the chloroform and acetone mixing test was inconclusive for this system, the bromothymol blue indicator test (Figure 9) indicates that a T-shape mixer provides efficient mixing of two solutions.

Estimation of the Flow Stop Time

High flow rates of solution through the probe result in significant line broadening of the NMR signal if the NMR excitation pulse occurs prior to flow stoppage. Therefore, the time for the solution to stop flowing (known as the stop time) must be determined to obtain quality spectra. To measure the stop time, the system was filled with toluene and a short delay prior to a 30° excitation pulse was varied. Zero time (time = 0) was defined as the time

when the pneumatic driver had pushed solvent into the system, hit the physical stop, and activated the optical trigger. At time = 0 the optical trigger signaled the spectrometer to start the pulse sequence. The stop time was determined by monitoring the width at half height ($w_{1/2}$) of the toluene methyl signal as measured at different delay times for three different flow rates. The stop time was estimated by noting the minimal delay needed to maintain narrow peak widths at half height (Figure 13).

As displayed in Figure 13, a 30 ms stop time is necessary to obtain protio solvent peak widths less than 50 Hz. A 100 ms stop time is required to obtain protio solvent peak widths less than 20 Hz, which is more relevant for reaction monitoring. The phenomenological stop-time represents the pulse delay required to obtain spectra of a given quality. It should be noted that a variety of factors influence the effective stop-time, including the time for vibrations of the detection coil and reactor tubes to ring down. Also, the tubing in the probe expands during high pressure flow and contracts after liquid flow slows down, contributing to movement of solution through the active region.

Estimation of the Optimal Solvent Push Volume

To test the optimal solvent push volume (the volume of reagent solution needed to completely displace the detection region with fresh reagents), the polymerization of lactide with catalyst IMes was performed using the following total solvent push volumes: 0.4, 0.5, 0.6, and 0.7 mL (Figure 14). Polylactide (PLA) was not observed at the first time point for each push volume. In addition, the reaction rate and final PLA concentration is the same for each push volume, indicating all the reacted solution is removed from the detection region. Therefore under these conditions, 0.4 mL total push volume is sufficient to completely replace the solution in the active region with unreacted reagent solutions; however, this push volume may vary with solvent choice, temperature, or viscosity of the final product solution.

Monitoring the Polymerization of Lactide at Variable Temperatures in Protio-toluene

The growth of PLA with time over the temperature range of -30 to 40 °C is displayed in Figure 15. These polymerizations were performed in protio toluene so the results display a low S/N ratio. However, reaction progression may be monitored even in protio solvent.

Experimental

General Experimental Methods

Stopped-Flow NMR was performed on a Bruker AV 360 MHz spectrometer. Toluene, THF, and hexanes were each distilled under N_2 from sodium benzophenone and stored over molecular sieves. Toluene-d_8 was stored over molecular sieves prior to use. IMes was synthesized according to literature procedure, except the deprotonation step was performed by stirring with NaH overnight, and recrystallized with THF/hexanes.[35] *Meso*-enriched lactide (89% *meso*-, 11% *rac*-lactide) was purchased from Natureworks LLC and dried over activated alumina. $Al(^iBu)_2$(BHT) (BHT = 2,6 di-tert-butyl-4-methylphenol) was synthesized according to the literature procedure.[36] 1,4-bis(trimethylsilyl)benzene (BTMSB) was sublimed and stored in a N_2 glovebox. Modeling was performed using Copasi software[37] with the Levenberg-Marquardt algorithm, an iteration limit of 2000, and a tolerance of 10^{-6}. The weighting method was mean square.

General Stopped-Flow Procedure

The stopped-flow procedure was previously described, and the procedure was followed as described except where noted.[28] Three syringes were prepared in an N_2 atmosphere glovebox: (a) *meso*-enriched lactide (43.4 mg, 0.301 mmol) and BTMSB (10.1 mg, 0.045 mmol) in 5 mL toluene-d_8, (b) IMes (3.00 mg, 9.88 μmol) in 5 mL toluene-d_8, (c)

$Al(^{i}Bu)_2(BHT)$ (110.6 mg, 0.308 mmol) in 5 mL toluene. The syringes were sealed in plastic bags in the glovebox and transferred to a N_2-purged glovebag. The drive system and NMR probe were filled with toluene and each reagent line was injected with 2.5 mL of syringe (c) and allowed to stand for 30 minu to remove trace water. 10 mL toluene was pushed through the system to remove $Al(^{i}Bu)_2(BHT)$ from the reactant lines. Syringes (a) and (b) were then simultaneously injected into the separate reagent lines. Each SF NMR run used 0.2 mL of each solution (0.4 mL total volume) unless otherwise noted. The delay prior to the 30° pulse was set to one second, acquisition time was 4 seconds, and the delay after acquisition was 0.5 seconds, unless otherwise noted. Thus spectra may be collected every 5.5 s. In order to collect data with a sampling frequency greater than 5.5 s, multiple runs are performed with different prepulse delay times. To obtain data for each second of the reaction, five runs would be performed with 0, 1, 2, 3, and 4 s prepulse delays with a 0 s post-acquisition delay, collecting 1 pulse spectra at 5 s intervals through the course of the reaction. Interleaving the data from each run allows reaction observation at intervals that would be otherwise unattainable due to incomplete relaxation between spectra.

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Author Contributions

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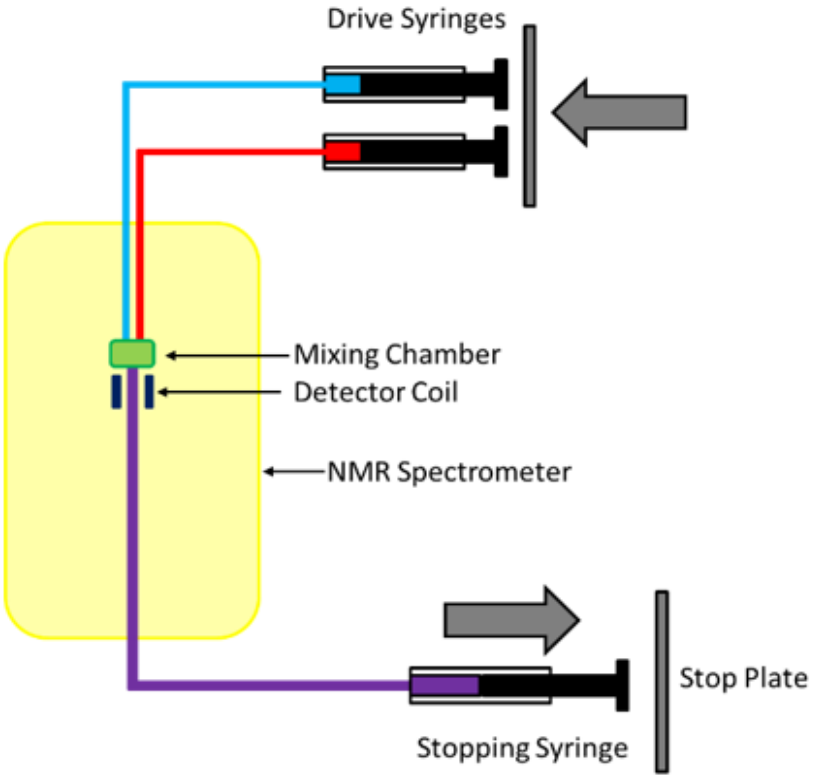


Figure 1. Generalized diagram of typical stopped-flow NMR instrumentation.

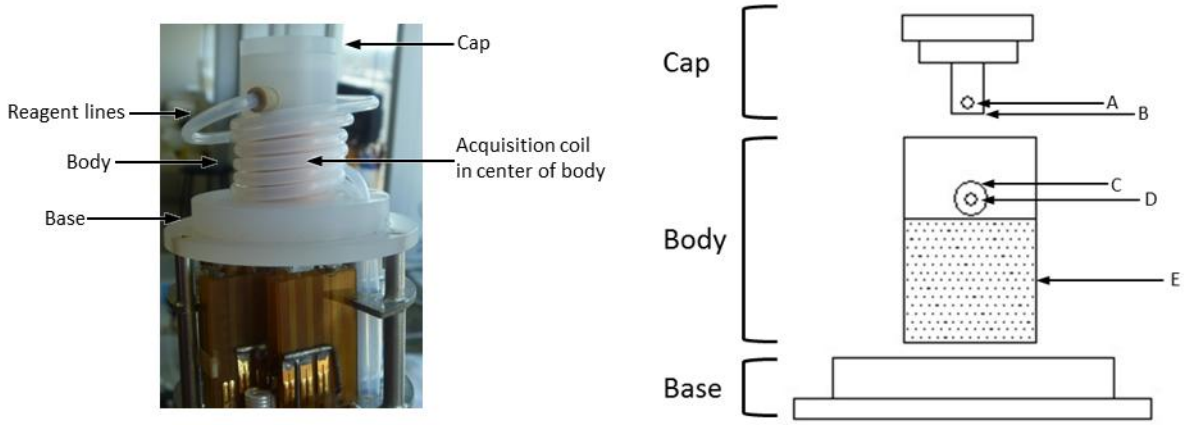


Figure 2. Photograph and diagram of the constructed SF NMR mixer. The cap inserts into the body such that the thru holes A and D line up to allow flow of each reagent stream into the mixer. There is a shallow indentation at the bottom of the cap (B) to allow for mixing of reagents. The reagent lines are screwed directly into the mixer at C. The dotted section (E) has spiraling guides for the reagent lines. The diameter of the cap and body is 1 inch.

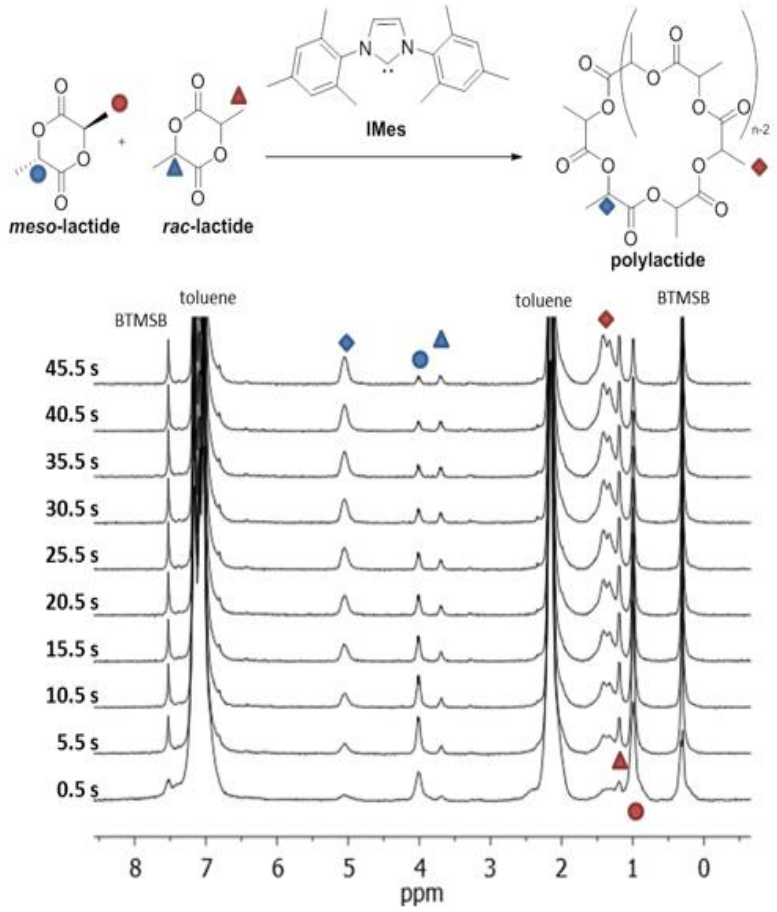


Figure 3. ¹H NMR spectra demonstrating the polymerization of *meso*-enriched lactide (30.1 mM) as catalyzed by IMes (1.0 mM) at 0 °C in toluene-d_8.

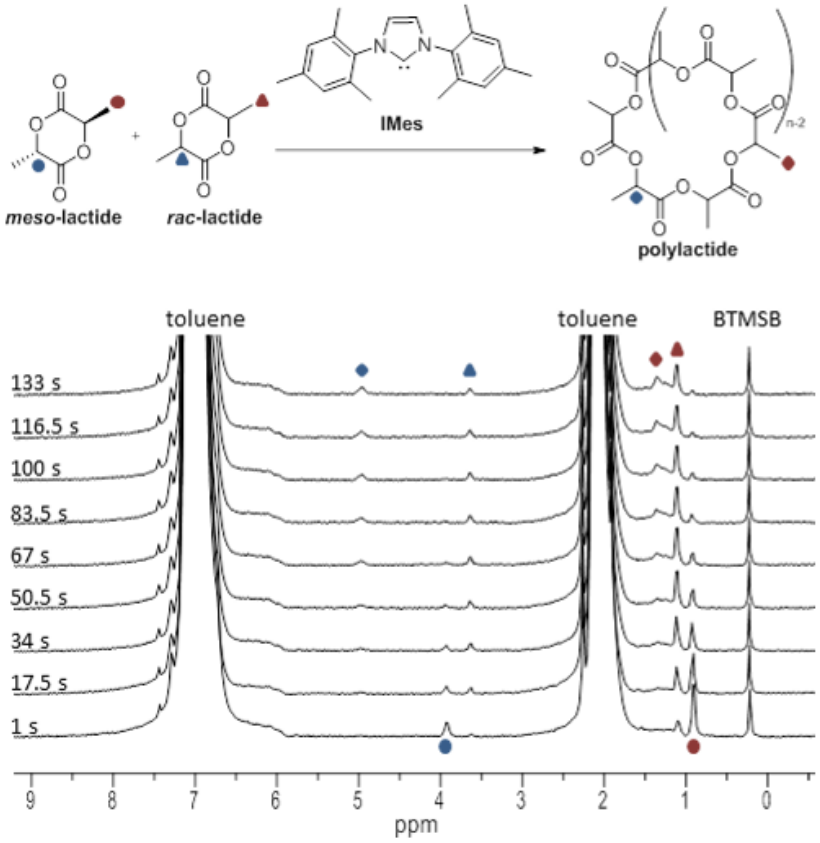


Figure 4. $_1$H NMR reaction progression of polymerization of *meso*-enriched lactide (29.8 mM) catalyzed by IMes (1.0 mM) at 0 °C in protio-toluene.

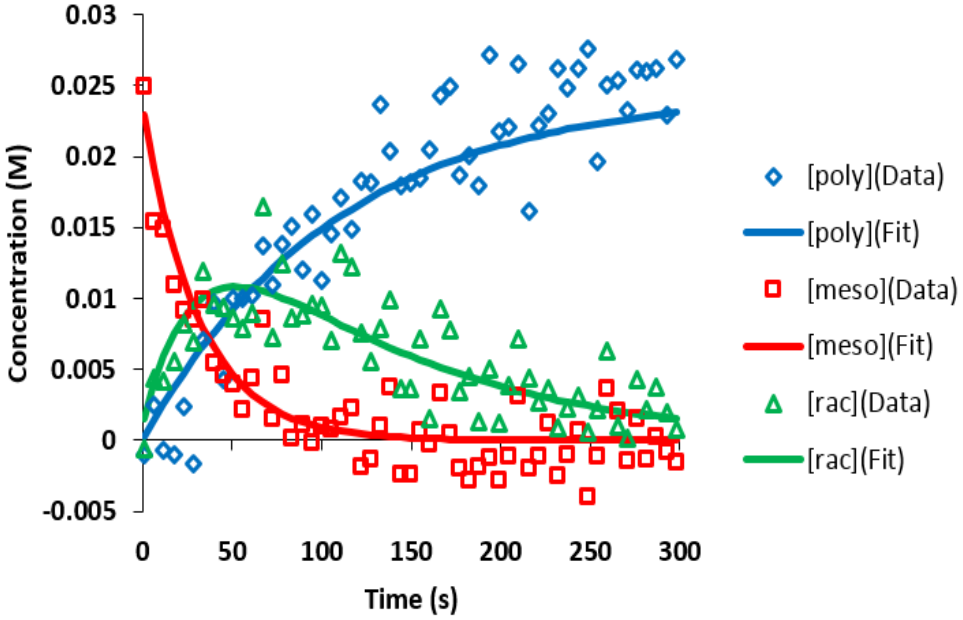


Figure 5. The fit (lines) of the data (points) to the kinetic model in Scheme 2 for lactide polymerization at 0 °C in protio toluene. [*meso*-lactide]$_0$ = 26.6 mM, [*rac*-lactide]$_0$ = 3.3 mM, [IMes]$_0$ = 1.0 mM.

Figure 6. Temperature equilibration of the reagent lines is achieved by placing a Dewar around the mixer.

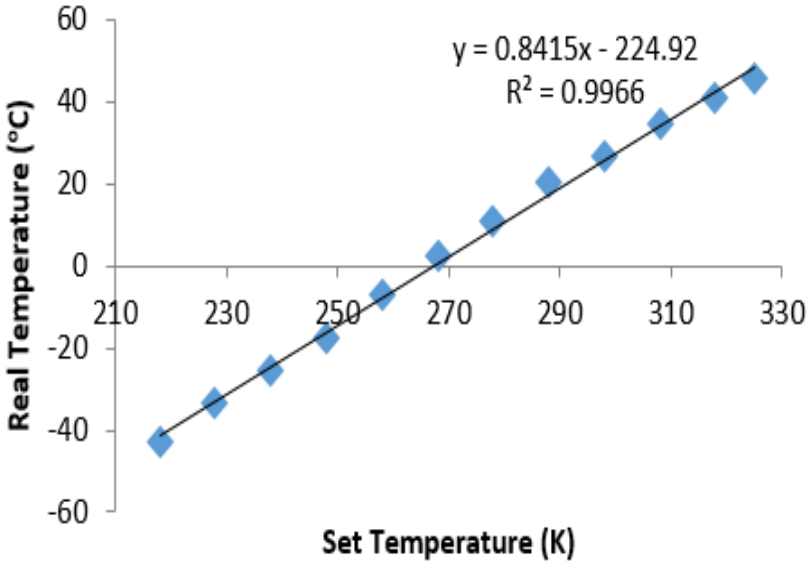


Figure 7. Calibration curve comparing the real temperature of the detection region as measured by the peak distance of pure methanol (°C) to the set temperature of the NMR spectrometer (K).

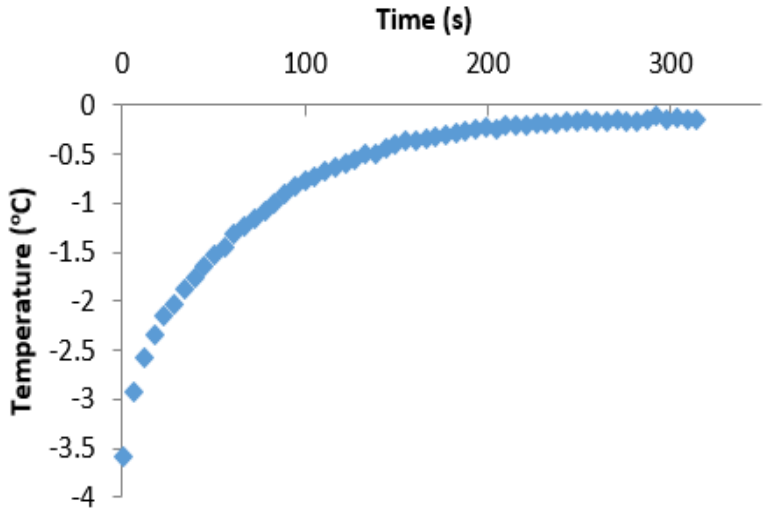


Figure 8. Temperature variation upon solvent push as measured by methanol $_1$H NMR chemical shifts. The spectrometer was set to regulate the temperature at 0 °C.

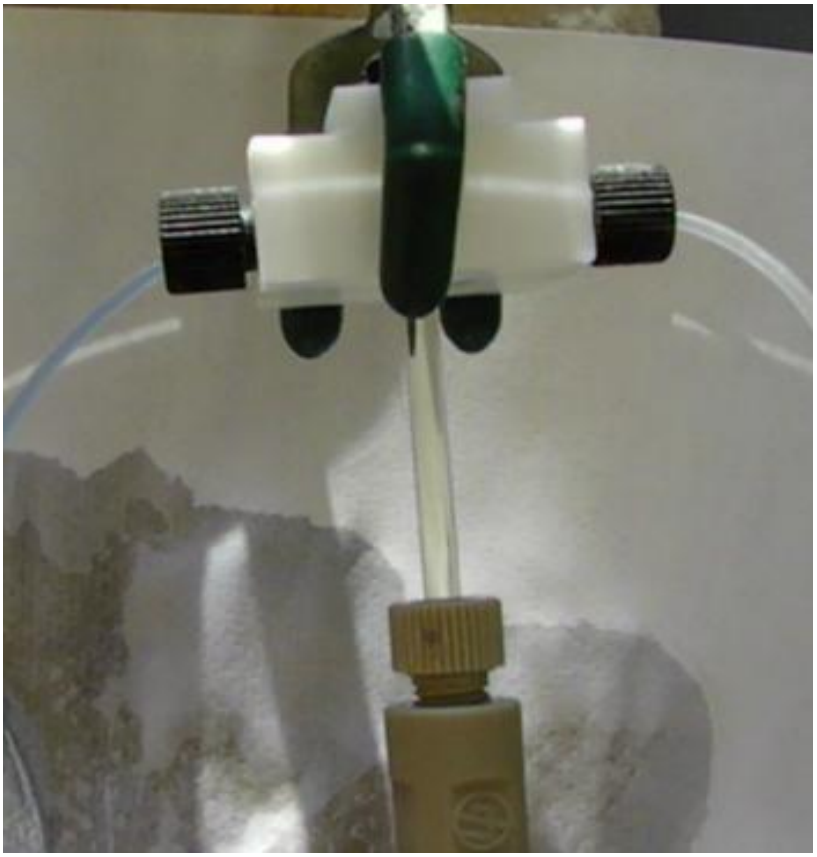


Figure 9. Photograph of the T-shaped mixer prototype. A basic solution with bromothymol blue indicator flows into the mixer from the left and an acidic solution flows into the mixer from the right. The absence of blue color from the mixed solution indicates complete mixing.

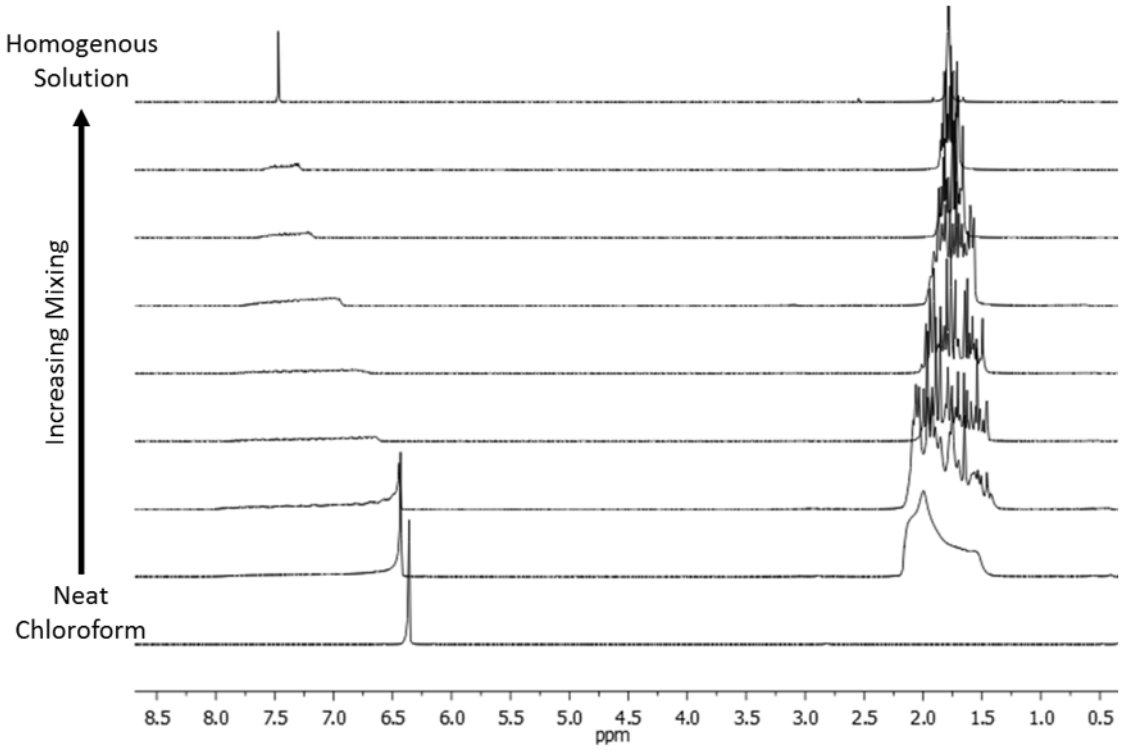


Figure 10. $_1$H NMR spectra of neat chloroform (bottom) which was layered with acetone (next) and increasingly mixed until a homogeneous solution (top) is formed. All spectra were acquired at 24 °C on a 500 MHz Bruker spectrometer and externally referenced to a neat acetone sample.

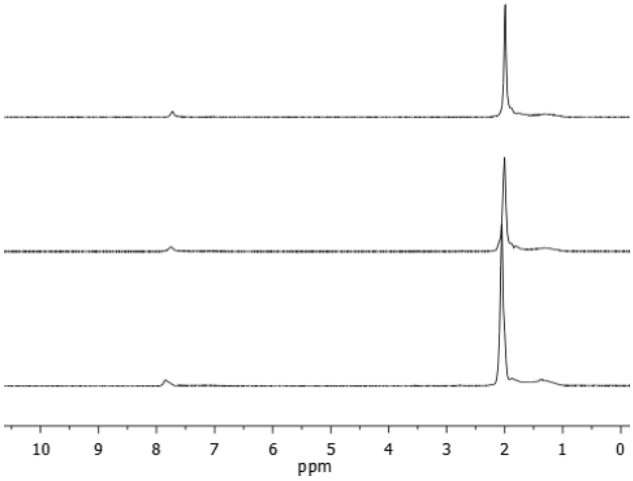


Figure 11. $_1$H NMR spectra after mixing of chloroform and acetone. The first spectrum (bottom) was acquired 1 s after mixing, each subsequent spectrum was acquired 2 s later.

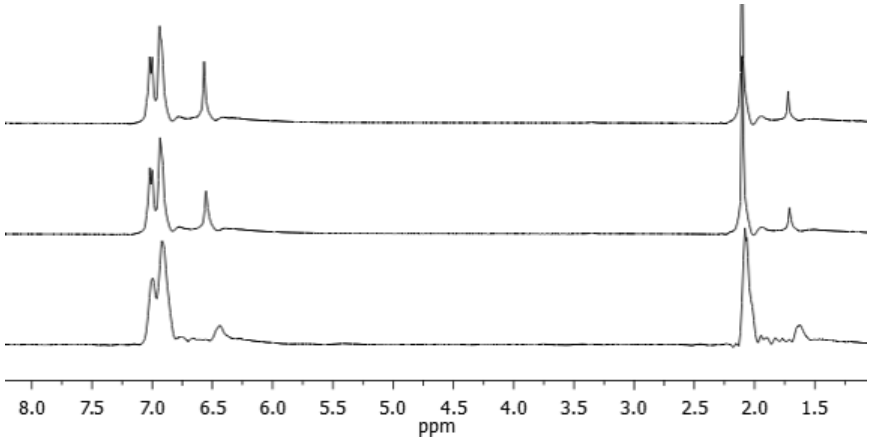


Figure 12. $_1$H NMR spectra after mixing chloroform and acetone. The first spectrum (bottom) was acquired 200 ms after mixing, each subsequent spectrum was acquired 14.2 s later.

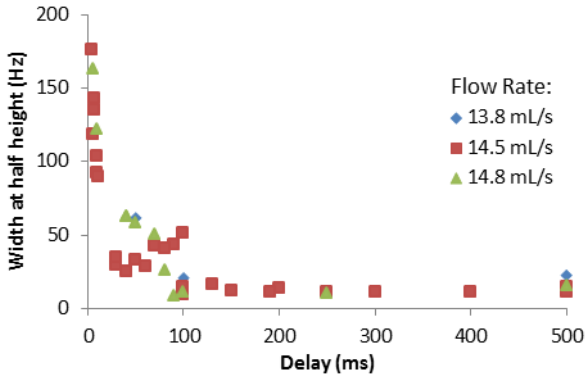


Figure 13. Estimation of the stop time by altering the delay prior to the 30° pulse and measuring the width of half height of the toluene methyl $_1$H NMR signal.

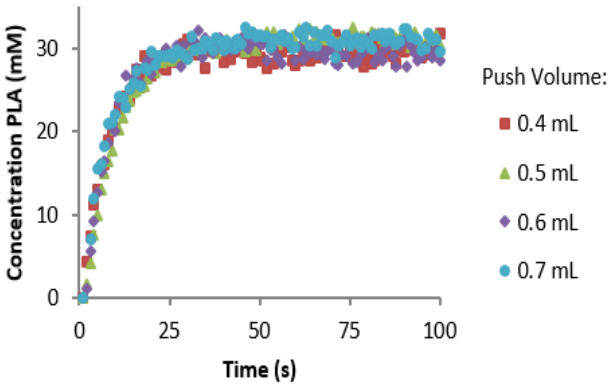


Figure 14. Growth of PLA with time for different solvent push volumes. Each reaction was performed at 0 °C in toluene-d_8 with [*meso*-enriched lactide]$_0$ = 30.1 mM, [IMes]$_0$ = 1.0 mM.

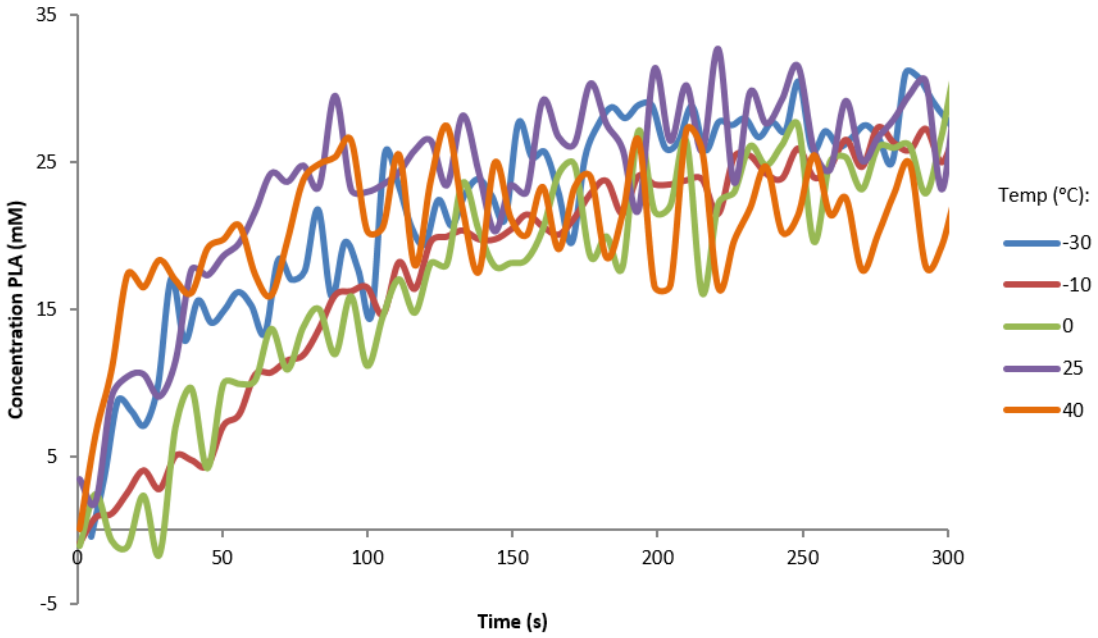
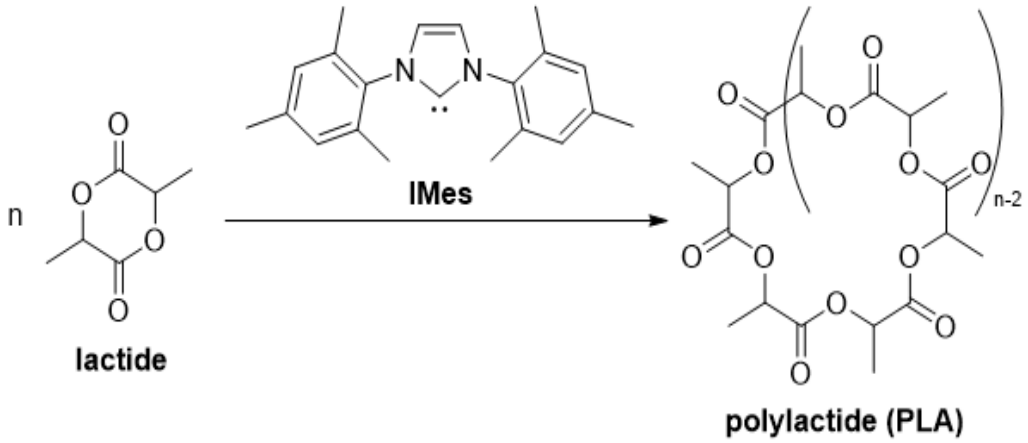
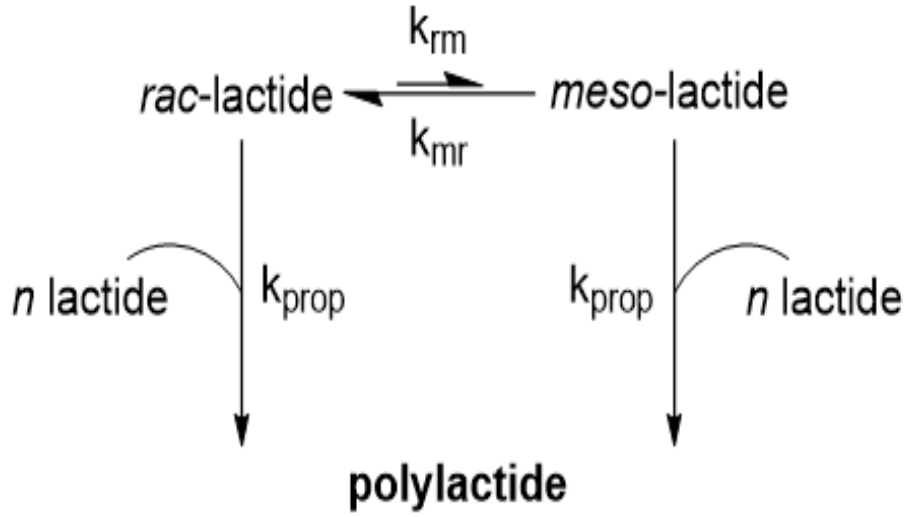


Figure 15. Growth of PLA with time at several different temperatures collected on the SF NMR probe. Concentrations obtained by comparison with internal standard BTMSB. All polymerizations were performed in protio toluene with the following initial concentrations: [*meso*-enriched lactide]$_0$ = 29.8 mM, [IMes]$_0$ = 1.0 mM.



Scheme 1. The polymerization of lactide to polylactide (PLA) by 1,3-dimesitylimidazol-2-ylidene (IMes).



Scheme 2. Simple kinetic model to simulate monomer epimerization and propagation.