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Reaction monitoring using online vs tube NMR spectroscopy: seriously different results

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We report findings from the qualitative evaluation of nuclear magnetic resonance (NMR) reaction monitoring techniques of how each relates to the kinetic profile of a reaction process. The study highlights key reaction rate differences observed between the various NMR reaction monitoring methods investigated: online NMR, static NMR tubes, and periodic inversion of NMR tubes. The analysis of three reaction processes reveals that rates derived from NMR analysis are highly dependent on monitoring method. These findings indicate that users must be aware of the effect of their monitoring method upon the kinetic rate data derived from NMR analysis. Copyright © 2015 John Wiley & Sons, Ltd.

Keywords: NMR reaction monitoring; online NMR; reaction mechanism

Introduction

Nuclear magnetic resonance (NMR) is an extremely powerful tool for the analysis of reaction mixtures. Not only can NMR be used as a quantitative method of monitoring reaction processes, it also has the advantage of providing detailed structural information about reaction components in solution. These two characteristics give NMR a distinct advantage over other analytical techniques typically used for reaction monitoring purposes. Some of these techniques (i.e. infrared or Raman) can identify functional groups of the molecules being analyzed; however, they lack the comprehensive characterization that NMR can provide.

There are four common approaches to conducting NMR reaction monitoring: (i) static, in standard NMR tubes, (ii) online monitoring, (iii) stopped-flow, and (iv) rapid injection NMR. Static NMR tube monitoring (i) is the simplest and therefore most extensively used of these techniques, and involves placing reagents into a standard NMR tube, then monitoring reaction progress of the static solution within the spectrometer.[1] This approach requires no specialized equipment beyond that normally used to run NMR samples and can be practically conducted in deuterated solvent because of the small volume required. This has been shown to be an effective method for probing mechanistic details of reactions.[2] An alternative method is to remove aliquots from a reaction vessel (sometimes followed by a quench step) and transfer the sample to an NMR tube for offline analysis.[3] This could equally be conducted in protonated solvents using proton gradient shimming for efficient shimming without the presence of a deuterium source.[4]

The second approach, online NMR (ii), transfers a flowing stream of the reaction mixture from a reaction vessel to the NMR probe where the analysis is conducted. The speed of flow is optimized to allow enough residence time of the magnetized sample for acquisition.[5] The reaction mixture can then be returned to the reaction vessel or directed to a waste container, depending on the requirements of the experiment. Online NMR requires special probes or custom NMR tubes, however it does facilitate online sampling, obviating the need for operator input once the experiment is running. Monitoring an online sample from a reaction vessel allows replication of reaction conditions while allowing for analysis in an essentially unperturbed state. There are examples of online NMR reaction monitoring being applied at both high and low fields.[6]

The third method, stopped-flow NMR (iii), is typically used for the detection of rapid kinetics (within 2.5–100 ms of reagents mixing), a feature that both NMR tube and online reaction monitoring cannot provide because of the time required to mix and get reagents to the detection region.[7] Stopped-flow NMR typically employs custom probes designed to flow reagents first into a mixing region and then into the detection region of the spectrometer with extremely high flow rates to minimize dead time. Prior to acquisition, the flow must be stopped because of the short residence times of magnetized sample at the high flow rates. The flow system allows for multiple time points to be easily taken by flowing more unreacted starting material into the mixing, then detection regions with different delay values in the pulse sequence. The stopped-flow NMR technique has allowed detection of short-lived intermediates, study of complex protein-folding mechanisms,[8] and insights into the effects of fast kinetics on NMR lineshape.[9]

The fourth approach, rapid injection NMR (iv), allows acquisition typically 40 ms–1 s after reagents have mixed. Rapid-injection NMR often utilizes custom probes or probe inserts that allow for quick insertion of reagents into the NMR tube and sometimes include a mechanical mixing period along with injection.[10] Rapid-injection NMR techniques have allowed direct observation of multiple reactive intermediate species, providing valuable mechanistic information.[11] However, the lack of a flow system means that subsequent

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measurements may only be taken after replacement or cleaning of parts of the system.

In the last few decades, there have been numerous reports on the analysis of reaction mixtures using NMR. The majority use standard NMR tube reaction monitoring.[12] While there is little doubt that monitoring a static NMR tube gives excellent mechanistic information, there is a lack of proof of how kinetic information gathered from this type of experiment is representative of how a reaction process proceeds under standard laboratory-scale synthetic chemistry conditions. We evaluated the validity of the following NMR reaction monitoring techniques from a kinetic standpoint: online NMR, standard NMR tubes, and NMR tubes with periodic inversion. The results that we present here demonstrate that reaction monitoring techniques have a significant influence on the kinetic understanding of a reaction.[13]

Results and discussion

This study was undertaken to evaluate how basic NMR tube monitoring techniques compare with following the progress of a reaction using an online NMR system such as the one developed in our laboratory.[14] While numerous reports of kinetic and mechanistic studies using both standard NMR tube and online approaches have appeared in the literature, none have undertaken a systematic comparison of NMR reaction monitoring techniques. This investigation was designed to look at both homogeneous and heterogeneous reactions, to provide evidence that the NMR monitoring technique can influence the kinetic results for a wide variety of reactions.

Three modes of NMR spectroscopy reaction monitoring were investigated; online NMR spectroscopy, NMR tube reaction monitoring with and without periodic agitation of the reaction mixture. The online NMR method, using our custom reaction monitoring system, was directly compared with the basic method of reaction monitoring in an NMR tube.[15] A third method was also used involving periodic inversion by inverting a standard NMR tube three times during the interval between each NMR spectrum acquisition. Periodic inversion gave insight into an intermediate mixing regime between constant mixing (online NMR) and a static sample (NMR tube).

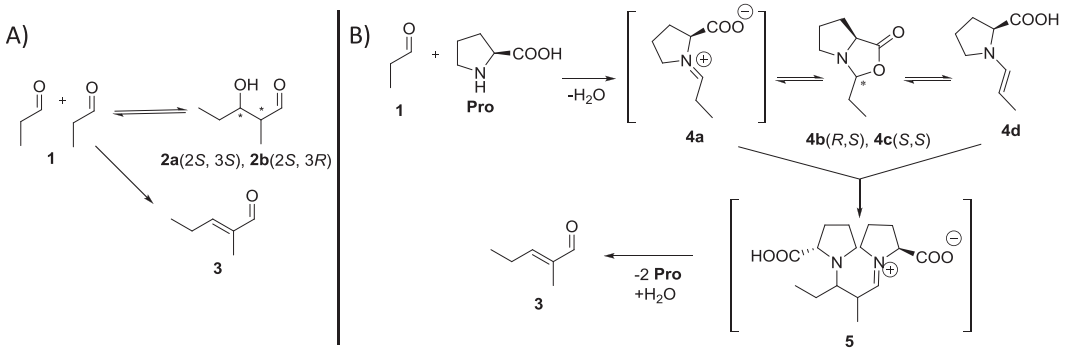
Three reaction types were evaluated in this study. The L-proline-catalyzed self-condensation of propionaldehyde was examined to compare different NMR methods of observing heterogeneous reaction progression. The homogenous coupling reaction of aniline and 4-fluorobenzaldehyde was also analyzed using the same three reaction monitoring methods (online, tube, and periodic inversion). Finally, the acid-catalyzed transesterification of isopropanol and acetic anhydride was also studied to show that the results obtained are not limited to only very slow reactions.

Heterogeneous reaction

The L-proline-catalyzed self-condensation of propionaldehyde **1** was chosen for the kinetic comparison study because of the well-established mechanism and the ability to observe many intermediate species via NMR spectroscopy.[16] In 2011, Zeitler and Gschwind reported an elegant mechanistic study of the L-proline-catalyzed self-condensation of propionaldehyde **1**. The conclusions of their study, which involved observing reaction progression in an NMR tube, indicated that the aldol addition pathway to **2a**/**2b** is competitive with aldol condensation to **3** (Scheme 1A), and the mechanism is a double-activation Mannich-type mechanism involving two catalyst molecules (Scheme 1B). According to their results, the mechanism involves simultaneous activation with proline (**Pro**) of the donor aldehyde to form **4a** and the acceptor aldehyde to form **4d**. The interconversion between **4a** and **4d** goes through **4b**/**4c**. The C–C bond formation step was indicated to be the rate determining step and could be monitored by observing the species **4b** – the maximum concentration of **4b** was determined to correspond with the fastest formation rate of **3**.

Mechanistic observations from this study were consistent with those reported by Zeitler and Gschwind, so all chemical assignments used in this study are in agreement with their published chemical shifts.

The progress of the L-proline-catalyzed aldol condensation was monitored by ^{1}H NMR by three methods; (i) online NMR, (ii) periodic inversion, and (iii) a static NMR tube. This heterogeneous reaction clearly displays the effect of efficient mixing on reaction progression. All three experiments were conducted at the same concentration (50 mM). The results of these experiments are displayed in Fig. 1. Characteristic resonances outlined by Gschwind *et al.* were also tracked as reaction monitoring handles.[16a] Because of overlap of the aldehyde protons related to **1** and **2a** at δ_H 9.65 ppm, these two components were tracked together. Intermediate **4b** was monitored using the resonance at δ_H 5.06 ppm and the aldol condensation product **3** at δ_H 9.34 ppm. It can be clearly seen from the reaction profiles that the time to reaction completion is very different when adequate mixing is applied. Intermediate **4b**, which was correlated with the rate determining step in Zeitler and Gschwind's study, forms at a much faster rate when agitation is



Scheme 1. (A) Aldol addition to form **2a**/**2b** indicated a competitive process with the desired aldol condensation to **3**. (B) Double-activation Mannich-type pathway indicated in the mechanistic study by Zeitler and Gschwind. Species in brackets could not be directly detected by NMR.

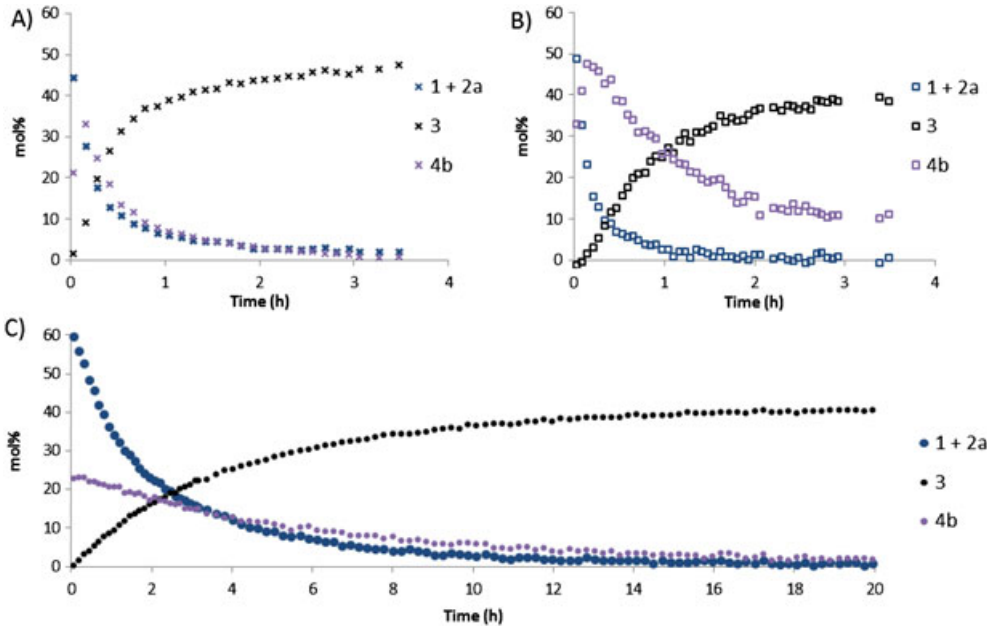


Figure 1. Study of the L-proline-catalyzed self-condensation of propionaldehyde **1** in dimethyl sulfoxide at 27 °C as observed by (A) online NMR, symbol ×; (B) NMR tube with periodic inversion, symbol □; (C) static NMR tube, symbol ●. All methods have initial **1** and **Pro** concentrations of 50.0 mM.

applied (Fig. 1A and B). When the reaction is diffusion controlled, as for the static NMR tube (Fig. 1C), there is much less **4b** observed, corresponding to the much slower rate of product **3** formation. Mol% was calculated by setting all propionaldehyde methyl signals and the methylene signal from the one intermediate without a distinct methyl (**4d**) from the first spectrum to 100%.

Figure 2 displays an overlay of the rate of product formation from all three methods (online NMR, static NMR tube, and periodic inversion) on a single plot, demonstrating clearly that rate of formation of product is dependent on amount of mixing. For clarity, minor components involved in the reaction mechanism (i.e. **2b**, **4b**, **4c,** and **4d**) are not shown; however, these were included in the mass balance calculation and conversion to mol%. The method with continuous mixing, online NMR, has the fastest growth of product. The method with an intermediate amount of mixing, periodic inversion – where there are some periods of mixing and some periods of no mixing – shows an intermediate rate of product growth. The NMR tube reaction, which is entirely under diffusion control, displays by far the slowest rate of product growth, clearly demonstrating that any kinetics derived from the reaction conditions are highly dependent upon the amount of mixing in the system and do not necessarily display the true kinetics of the reaction under investigation. In addition, variability in the rates derived from the NMR tube monitoring was observed, likely because of the inability to control how much catalyst stuck to the walls of the NMR tube upon the initial mixing of reagents.

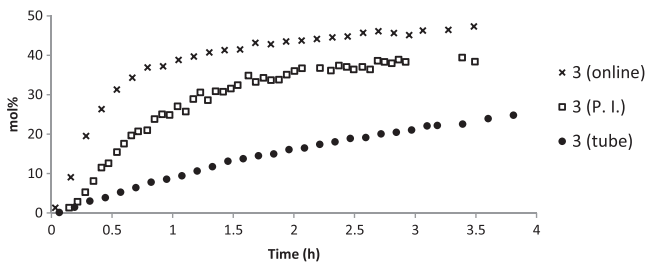
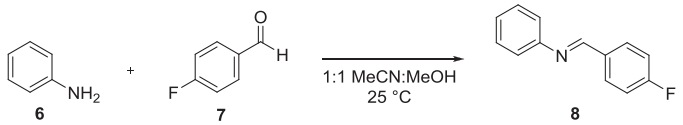


Figure 2. Overlay of product formation profiles for all methods used to study the L-proline-catalyzed self-condensation of propionaldehyde **1** to form **3** in dimethyl sulfoxide at 27 °C.

Homogenous reaction

The second reaction investigated was the homogeneous coupling reaction of aniline **6** and 4-fluorobenzaldehyde **7** to produce the corresponding imine at 25 °C (Scheme 2). This reaction was chosen for the investigation as a comparative study to the heterogeneous case, outlined in the previous section. The reaction kinetics were found to be relatively slow, and ^{19}F NMR spectroscopy could be used to monitor the progress of the reaction.

The results from this experiment are outlined in Fig. 3. There is a less pronounced difference between the kinetic results obtained (online vs tube) when monitoring the homogeneous reaction as compared with the heterogeneous reaction. Mol% was calculated by setting the total amount of 4-fluorobenzaldehyde **7** and imine **8** detected by ^{19}F NMR at each time point to 100% (at -105.2 and −110.2 ppm, respectively). The method with the most mixing (online NMR) has decidedly the quickest conversion to product. Periodic inversion of the NMR tube does not increase the rate of reaction as compared with the static NMR tube, showing that for this



6 7 8

Scheme 2. The coupling reaction of aniline **6** and 4-fluorobenzaldehyde **7** to form imine **8** in 1 : 1 methanol: acetonitrile at 25 °C.

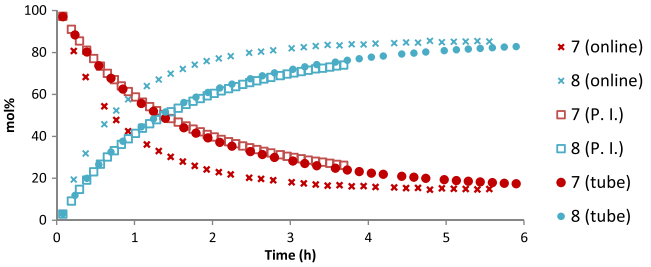


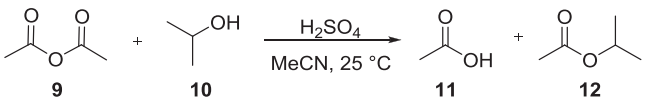
Figure 3. A summary of all methods used to study the aniline **6** and 4-fluorobenzaldehyde **7** coupling reaction to form the imine **8** in 1 : 1 methanol: acetonitrile at 25 °C. Online nuclear magnetic resonance (NMR) (×) initial concentrations were 64 mM each of **6** and **7**. Periodic inversion (□) and NMR tube (●) reactions' initial concentrations were 69 mM each of **6** and **7**.

homogenous reaction, inversion every 6–7 min is not enough to show improvement upon diffusion-limited mixing. Regardless, it is clear that any kinetic rates derived from the static NMR tube would reflect more upon being diffusion controlled than the actual rates of reaction.

Homogeneous reaction, fast kinetics

In order to test the effect of mixing on reactions with shorter half-lives (i.e. on the order of a few minutes), the transesterification of acetic anhydride **9** and isopropanol **10** to produce acetic acid **11** and isopropyl acetate **12** at 25 °C in acetonitrile was studied (Scheme 3).

Figure 4 shows that even for reactions with half-lives on the order of minutes, mixing is still crucial to obtaining accurate rate values. The periodic inversion results for the transesterification reaction are not presented because of the reaction completion being too fast to obtain an appropriate number of data points. Mol% was calculated by setting the total integration of isopropanol **10** methine (3.9 ppm) and isopropyl acetate **12** methine (4.9 ppm) detected at each time point to 100%. Because of the residence time characteristics of the online system used, the early portion of the online data (<3.2 min) cannot be used for kinetic calculations; however, there is still a clear difference in the reaction completion time between the two experiments. This difference is again due to the mixing effects,



Scheme 3. The sulfuric acid-catalyzed transesterification reaction of acetic anhydride **9** and isopropanol **10** in acetonitrile at 25 °C.

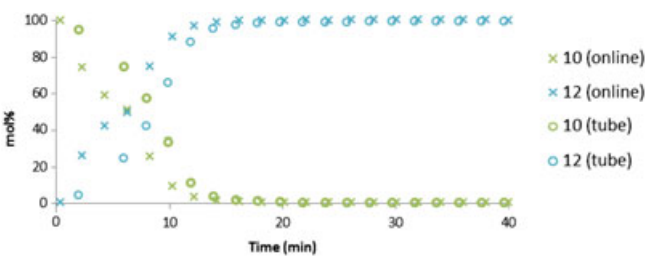


Figure 4. A summary of the methods used to study the transesterification reaction of acetic anhydride **9** and isopropanol **10** in acetonitrile at 25 °C. Online NMR initial concentrations were 2.5 M each of **9** and **10** and 44 mM H_2SO_4. Tube initial concentrations were 2.3 M each of **9** and **10** and 46 mM H_2SO_4.

efficient stirring is applied in the reaction vessel in the case of the experiment monitored by online NMR spectroscopy, while diffusion-controlled kinetics have a greater effect on the result obtained from the static NMR tube experiment.

There are positive and negative aspects to each reaction monitoring technique that must be evaluated when designing an experimental procedure. Table 1 summarizes some of these considerations. Online NMR monitoring allows the closest replication to a non-monitored experiment, achieving control over parameters such as temperature, rate of mixing, and rate of addition of reagents, which is key to developing a chemical reaction process. There are some limitations associated with monitoring a reaction online because of the distance between the reaction vessel and the detection cell, which can influence sample transfer time and temperature gradients. Details of the online NMR spectroscopy system used in this study have been published previously,[14] and a number of reports of the characteristics of online NMR systems have provided in-depth analysis of the considerations when conducting this type of analysis.[5,6b,17] Online NMR also allows for the introduction of other analytical instruments in-line, but requires specialized equipment that may not be available to all users.

Conducting a reaction in an NMR tube requires only basic equipment, but at the cost of sacrificing efficient mixing during the reaction. Temperature gradients are likely between the top and bottom of the NMR tube, especially at elevated temperatures, also evaporation and condensation of volatile solvents could be experienced, which could result in concentration gradients or changes because of solvent loss. These effects will influence the kinetic results obtained.

The periodic inversion method also requires only basic equipment; however, it has the major drawback of the operator having to physically invert the NMR tube between each spectrum for the entirety of the reaction. Acquiring data points in this way can also lead to brief loss of temperature control of the sample, which can affect the results obtained. Taking aliquots from a stirred reaction vessel should give similar results to a non-monitored experiment, but again requires the operator to be present at each time point. Aliquots of the L-proline-catalyzed aldol condensation were also taken, but upon diluting the reaction mixture with deuterated solvent to lock, the sample was far too dilute to get meaningful results. Also, taking aliquots causes brief loss of temperature control as well as loss of material.

One must also consider the larger purpose of the NMR monitoring study when deciding upon a monitoring method. If the data from the monitoring study are to be used in the development of a chemical process for scale-up, it would be wise to use a monitoring method that facilitates similar reaction conditions to the large-scale reaction, such as that provided by online NMR.[18] Online NMR is particularly amenable to tracking flow chemistry processes,

Table 1. Summary of some important considerations when choosing an NMR monitoring method

Conditions	Online NMR	NMR tube	Periodic inversion
Automated sampling	✓	✓	✗
Temperature control	✓	✓	✗
Efficient mixing	✓	✗	✗
Standard equipment	✗	✓	✓
Deuterated solvent	✗	✓	✓
Scalability	✓	✗	✗

NMR, nuclear magnetic resonance.

where the output stream can be readily monitored in real-time, allowing quick optimization of experimental conditions. Offline sampling will not produce accurate results for a flow chemistry process as a result of averaged response because of pooling of the sample stream in the detection cell before acquisition. Accurate results would require the use of a flow-through NMR cell, rather than the online NMR tube system used to monitor the reactions described in the current study, because of similar pooling effects.

Conclusion

In conclusion, we present evidence that mixing has a large effect on the rate of reaction determined by NMR spectroscopy for three different types of reactions [heterogeneous, homogeneous with long (>1 h) reaction times, and homogeneous with short (<1 h) reaction times]. All three reactions studied show conclusive evidence that the NMR monitoring technique can have a significant effect on reaction rates, providing support for the application of continuous flow online NMR methods for kinetic studies when the most accurate results are required. Studies in static NMR tubes can provide good mechanistic and structural information particularly for labile or reactive intermediates. However, as the results from this work demonstrate, caution should be applied when relying on kinetic data acquired from systems lacking adequate mixing.

Experimental

General procedure for online NMR

The online NMR setup was used as previously described with removal of the needle splitting valve.[15] Temperature control was achieved by setting reaction vessel, sample loop, and spectrometer to the specified temperature. The flow rate was set to 4 ml/min.[14] Spectra were acquired on the flowing sample on a Bruker 400 MHz Avance III NMR (Billerica, MA, USA) equipped with broad band fluorine observation (BBFO) probe. ^{1}H NMR spectra were acquired with four scans, a 30° pulse angle, and a 10 s relaxation delay, unless otherwise noted. If applicable, ^{19}F{^{1}H} spectra were acquired with eight scans, 90° pulse angle, and 30 s relaxation delay.

General procedure for static NMR tube reactions

Reagents were added to a 5 mm NMR tube, inverted three times, and inserted into spectrometer that began taking spectra immediately. Spectra were acquired on a Bruker 400 MHz Avance III NMR equipped with BBFO probe. The temperature of the sample was controlled using the variable temperature heater of the probe. ^{1}H NMR spectra were acquired with four scans, a 30° pulse angle, and a 10 s relaxation delay, unless otherwise noted. If applicable, ^{19}F{^{1}H} spectra were acquired with eight scans, a 90° pulse angle, and a 30 s relaxation delay.

General procedure for NMR tube reactions with periodic inversion

Reagents were added to a 5 mm NMR tube, inverted three times, and inserted into spectrometer where initial spectrum was acquired. Prior to each subsequent spectrum, the NMR tube was removed from the spectrometer and inverted three times before inserting back into the spectrometer. Spectra were acquired on a Bruker 400 MHz Avance III with BBFO probe. The temperature of the sample was controlled by the variable temperature heater of the probe. ^{1}H NMR spectra were acquired with four scans, 30° pulse angle, and 10 s relaxation delay, unless otherwise noted. If applicable, ^{19}F{^{1}H} spectra were acquired with eight scans, 90° pulse angle, and 30 s relaxation delay.

L-proline-catalyzed aldol self-condensation of propionaldehyde at 27 °C

Online NMR. L-proline (**Pro**), (Amresco) (208.9 mg, 1.8 mmol) was suspended in 36 ml protio dimethyl sulfoxide (DMSO) in a 50 ml reactor, and propionaldehyde **1** (Sigma–Aldrich) (0.13 ml, 1.8 mmol) was added in one portion via syringe. ^{1}H NMR spectra were acquired at intervals of 5 until 200 min, then at 10 min intervals with a 90° pulse angle and a 15 s relaxation delay. The DMSO singlet was suppressed using the WET (water suppression enhanced through T1 effects) solvent suppression pulse sequence.[19]

Static NMR tube reaction. L-proline (**Pro**) (3.7 mg, 0.03 mmol) was suspended in 0.6 ml DMSO-d6 in an NMR tube. Propionaldehyde **1** (2.2 µl, 0.03 mmol) was added in one portion via micropipette, and the tube was inverted three times. ^{1}H NMR spectra were initially acquired at 5 min intervals, then at 10 min intervals. All spectra were acquired with eight scans and a 15 s relaxation delay.

NMR tube with periodic inversion. L-proline (**Pro**) (3.7 mg, 0.03 mmol) was suspended in 0.6 ml DMSO-d6 in an NMR tube. Propionaldehyde **1** (2.2 µl, 0.03 mmol) was added in one portion via micropipette. ^{1}H NMR spectra were acquired with eight scans.

Aniline and 4-fluorobenzaldehyde coupling at 25 °C

Online NMR. 4-Fluorobenzaldehyde **7** (Sigma–Aldrich) (0.28 ml, 2.6 mmol) was added to 20 ml protio acetonitrile and 20 ml protio methanol in a 50 ml reaction vessel. Aniline **6** (Sigma–Aldrich) (0.24 ml, 2.6 mmol) was added in one portion via micropipette. ^{19}F{^{1}H}, and ^{1}H NMR spectra were acquired at 5 min intervals.

Static NMR tube reaction. 4-Fluorobenzaldehyde **7** (7 µl, 0.07 mmol) was added to an NMR tube with 0.5 ml methanol-d4 and 0.5 ml acetonitrile-d3 via micropipette. Aniline **6** (6 µl, 0.07 mmol) was added in one portion via micropipette, and the NMR tube was inverted three times. ^{19}F{^{1}H} spectra were acquired at 5 min intervals. ^{1}H NMR spectra were also acquired but not used for analysis.

NMR tube with periodic inversion. 4-Fluorobenzaldehyde **7** (7 µl, 0.07 mmol) was added to an NMR tube with 0.5 ml methanol-d4 and 0.5 ml acetonitrile-d3 via micropipette. Aniline **6** (6 µl, 0.07 mmol) was added in one portion via micropipette. ^{19}F{^{1}H} spectra were acquired for analysis.

Isopropanol and acetic anhydride transesterification at 25 °C

Online NMR. Acetic anhydride **9** (Sigma–Aldrich) (10 ml, 106 mmol) and isopropanol **10** (Sigma–Aldrich) (8.1 ml, 106 mmol) were dissolved in 25 ml protio acetonitrile in a 50 ml reaction vessel. Concentrated sulfuric acid (J.T. Baker) (100 µl, 1.9 mmol) was added in one portion via syringe. ^{1}H NMR spectra were acquired in 60 s intervals.

Static NMR tube reaction. Acetic anhydride **9** (0.2 ml, 2 mmol), isopropanol **10** (0.16 ml, 2 mmol), and acetonitrile-d3 (0.5 ml) were added to an NMR tube. Concentrated sulfuric acid (2 µl, 0.04 mmol) was added in one portion via syringe. ^{1}H NMR spectra were acquired at 60 s intervals.

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