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A detailed mechanistic investigation into the reaction of 3-methylpentanoic acid with Meldrum's acid utilizing online NMR spectroscopy

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A thorough investigation into the mechanism of the reaction of 3-methylpentanoic acid and Meldrum's acid using online NMR spectroscopy is reported. This study is an expansion of a previous analysis of this chemical transformation in the synthesis of an active pharmaceutical ingredient imagabalin. The 3-methylpentanoic acid analogue reveals similar behavior under the reaction conditions. Online NMR spectroscopy and offline characterization experiments reveal new information about the mechanism, providing conclusive spectroscopic evidence for the previously hypothesized dimer anhydride intermediate species 3-methylpentanoic anhydride as a productive intermediate. The presence of an acyl chloride intermediate species, 3-methylpentanoyl chloride, is also revealed for the first time in this synthesis. Copyright © 2015 John Wiley & Sons, Ltd.

Keywords: NMR reaction monitoring; online NMR spectroscopy; reaction mechanism

Introduction

Process analytical technology is routinely used to monitor chemical reactions; however, most monitoring methods require some type of calibration step to normalize the output to relate concentration *versus* time.[1] Many of these instruments may not readily reveal which species in the reaction matrix are responsible for a given change in analytical response, because of their lack of structural information. NMR spectroscopy overcomes these limitations as it inherently provides a quantitative signal response based on the number of nuclei, and it is the primary structural elucidation tool for small molecule characterization, making it a powerful investigational tool for reaction understanding.[2]

Online NMR spectroscopy typically involves transfer of a reaction mixture from a reaction vessel to the active region of the NMR probe for measurement on the flowing sample without the need for discrete sampling or exposure to the external atmosphere. Multiple designs of online systems have been reported, which take advantage of the ability to track reaction progression in real time with minimal disturbance to the reaction matrix.[3] The application of online NMR has been used to probe reaction processes and monitor the progress of reactions in various fields.[4]

As part of the development of an active pharmaceutical ingredient,[5] a study of one of the steps in the synthetic route was undertaken by Clegg *et al.* (Scheme 1).[6] The reaction analysis was conducted by NMR spectroscopy, as other analytical techniques such as ultraviolet spectroscopy and gas chromatography–mass spectrometry (GC–MS) were not amenable to track reaction progression. The investigation by Clegg *et al.* revealed a mechanistic pathway involving pivalic anhydride **5** and mixed anhydride **6a** intermediates, and their structures were assigned based on two-dimensional (2D) NMR spectroscopy, GC–EI–MS and infrared spectroscopy data. However, additional peaks observed in the ^{1}H NMR spectrum suggested additional intermediate species were present in the reaction mixture. The authors also hypothesized that other intermediates could be chemically possible, such as 3-methylhexanoic anhydride **7a**, but no empirical evidence for their existence was noted during their study.

In the current work, we examine the analogous reaction using a shorter chain carboxylic acid **1b** (Scheme 2) and demonstrate the use of online NMR spectroscopy for reaction monitoring and mechanistic characterization of the transformation. All reactive intermediates are characterized by ^{1}H and ^{13}C NMR spectroscopy and their mechanistic pathways elucidated.

Results and discussion

Online NMR reaction profiling

In the original NMR study by Clegg *et al.*, the disadvantage of monitoring the progress of the reaction in an NMR tube was

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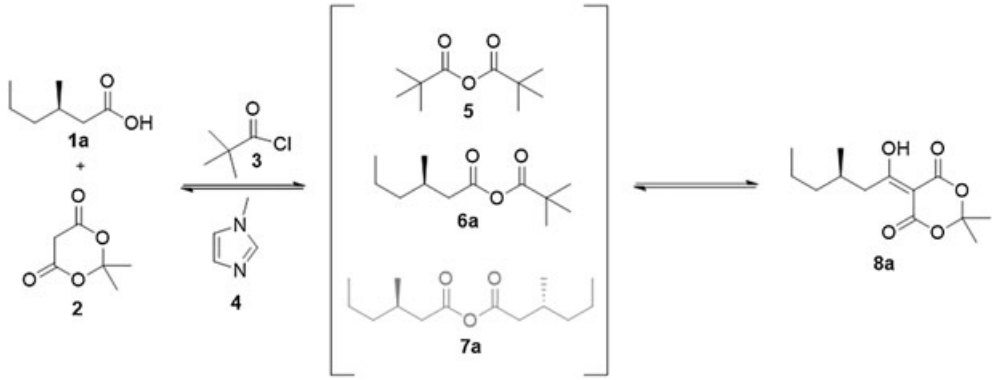
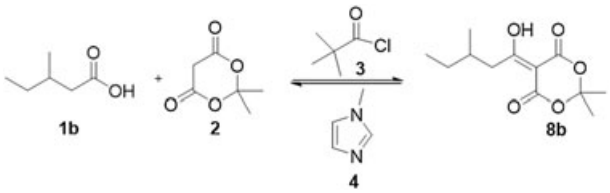


Scheme 1. Summary of the finding from the NMR study by Clegg *et al.* on the formation of **8a**, an intermediate in the synthesis of Imagabalin. The gray structure **7a** represents an intermediate that was hypothesized but not substantiated by evidence.



Scheme 2. Reaction scheme of the transformation studied using online NMR spectroscopy.

highlighted. Initiation of the reaction by dropwise addition of pivaloyl chloride **3** outside of the spectrometer, in addition to operations such as locking and shimming, caused the initial part of the reaction to be unobservable by NMR.

In the current study, the reaction displayed in Scheme 2 was analyzed using online NMR spectroscopy to assist in capturing the onset of reaction, providing a more complete reaction profile. Offline characterization of the unknown components within the reaction matrix was also conducted, allowing definitive mechanistic elucidation.

The online NMR spectroscopy experimental setup has been described previously and was designed to allow the chemistry to be conducted following the conditions under which the reaction would occur in the absence of NMR analysis.[3c,7] Therefore, reaction parameters such as agitation, dosing, temperature and atmosphere were easily replicated. These features provide distinct advantages over offline sampling or conducting the reaction in a standard NMR tube.

3-Methylpentanoic acid **1b**, Meldrum's acid **2** and 1-methylimidazole **4** were dissolved in acetonitrile in a 50 ml reaction vessel. The reaction solution was circulated throughout the system to achieve equilibration of concentration and temperature (25 °C). Pivaloyl chloride **3** was then added using a dosing syringe over the course of 10 min, while the online system was circulating at a rate of 3 ml/min. ^{1}H NMR spectra were obtained on the flowing reaction mixture at 2 min intervals for the first 20 spectra, at 6 min intervals until the reaction time reached 185 min and then at 16 min intervals until reaction completion. ^{1}H NMR spectra stacked plot of the aliphatic region of the initial 300 min after reaction initiation is displayed in Fig. 1a. The bottom spectrum in the stacked plot was recorded as the dosing of pivaloyl chloride into the reaction vessel began, but appearance of intermediates does not occur until the third spectrum (3.9 min reaction time) because of the transfer time from the reaction vessel to the NMR detection region and time delay between experiments.

A number of components were involved in the reaction, and key resonances that represent these components are displayed in Fig. 1b (a single ^{1}H NMR spectrum extracted from the stacked plot at 13.7 min after reaction initiation). The reaction profile is plotted in Fig. 1c, showing the change in reaction species concentration over the course of the reaction.

Intermediate characterization experiments

A number of species (labeled **A**, **B** and **C**, Fig. 1) were observed in the reaction matrix, and their structural identification was desired. While parallels with the 3-methylhexanoic acid **1a** reaction studied previously were likely, definitive proof of structure of the components observed in our experiment was desired. To elucidate these reaction components, a number of experiments were conducted to prove the structure of these unknown species. The design of these experiments was assisted by the knowledge gained in the investigation by Clegg *et al.* In their NMR tube study, resonances in the methyl region were used as characteristic signals for tracking the reaction components. The authors did not focus on the region between 2.0 and 3.5 ppm, which in our investigation was observed to contain a number of signals beneficial to reaction monitoring (Fig. 1b). To provide structural characterization of intermediate species **A**, **B** and **C**, experiments were conducted in deuterated acetonitrile in 5-mm NMR tubes. These experiments were designed to maximize the formation of possible intermediates to allow chemical shift and structure assignment.

Identification of anhydride 7b and acyl chloride 9

The first of these structural characterization experiments was carried out to verify that the dimeric anhydride, analogous to **7a** postulated by Clegg *et al.*, was present as an intermediate in the process. A sample of the dimeric anhydride **7b** was synthesized and characterized *in situ* in order to determine if this component was present and to identify which of the signals (**A**, **B** or **C**) it represented. The reference sample of the dimeric anhydride **7b** was generated in a stepwise manner via reaction of 3-methylpentanoic acid **1b** with oxalyl chloride in a 5-mm NMR tube to activate the carboxylic acid by conversion to the corresponding acyl chloride **9**. This was then reacted with a second mole of 3-methylpentanoic acid **1b**, furnishing the dimeric anhydride **7b**. 3-Methylpentanoic acid **1b** and *N*,*N*-dimethylformamide (DMF) are shown in Fig. 2a, confirming that the signals at 2.08 and 2.29 ppm belong to the starting material **1b**. Figure 2b presents the ^{1}H NMR spectrum of

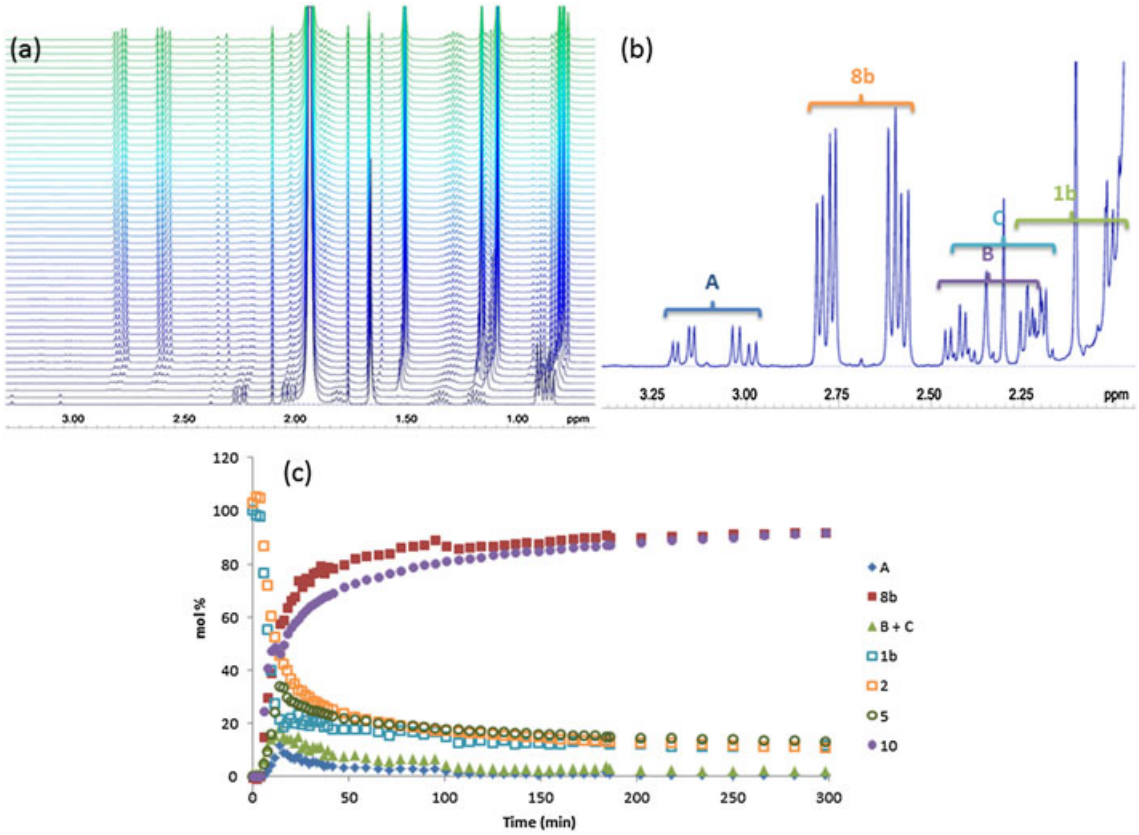


Figure 1. (a) ^{1}H NMR stack plot of the aliphatic region showing reaction progression (Scheme 2) with respect to time. The bottom spectrum is at time zero, with each subsequent spectrum spaced approximately 2 min (first 20 spectra) or 6 min apart (additional spectra). (b) 2.0–3.5 ppm region of the ^{1}H NMR spectrum recorded at 13.7 min reaction time. Characteristic ^{1}H NMR resonances of reaction components are highlighted. (c) Reaction profile for the reaction of 3-methylpentanoic acid with Meldrum's acid.

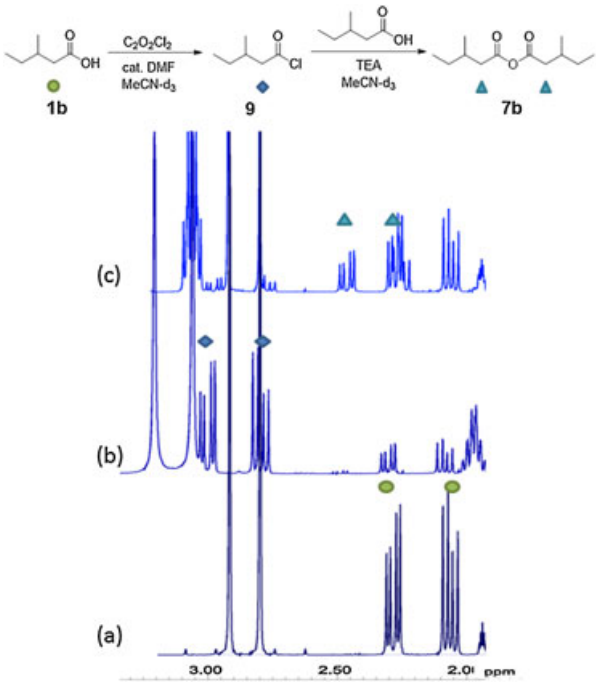


Figure 2. ^{1}H NMR spectral overlay of the two-step process to form the dimeric anhydride **7b** in acetonitrile-d_3. (a) ^{1}H NMR spectrum of 3-methylpentanoic acid **1b** and catalytic DMF; (b) ^{1}H NMR spectrum following the addition of one equivalent oxalyl chloride to the reaction mixture; and (c) ^{1}H NMR spectrum following the addition of 1.5 additional equivalents **1b** and triethylamine to the reaction mixture.

the reaction mixture obtained following addition of oxalyl chloride to 3-methylpentanoic acid **1b** in the presence of a catalytic amount of DMF, allowing for the identification of the signals at 2.77 and 2.98 ppm as 3-methylpentanoyl chloride **9**.

Comparison of spectrum (b) in Fig. 2 to the spectra recorded for online process monitoring revealed the acyl chloride **9** to be intermediate species **A** (Fig. 1b). This proved to be a valuable experiment in forming a reference sample for characterization of the pentanoyl chloride **9**, which was not previously indicated as being involved in the reaction mechanism in the case of the longer chain carboxylic acid (Scheme 1). With characterization of the pentanoyl chloride **9** complete, an additional 1.5 equivalents of 3-methylpentanoic acid and triethylamine were added to this sample. This resulted in formation of the dimeric anhydride **7b**, evident from a set of diastereotopic methylene protons at 2.25 and 2.46 ppm in the ^{1}H NMR spectrum shown in Fig. 2c. This independent synthesis of a reference sample of dimeric anhydride **7b** and observance of the same peaks in the reaction monitored by online NMR provided evidence that the dimeric anhydride **7b** was in fact an intermediate in the reaction (species **C**, Fig. 1b), lending evidence to the hypothesis proposed by Clegg *et al.*

Identification of mixed anhydride 6b

Clegg *et al.* identified the mixed anhydride species **6a** in their study of the reaction with 3-methylhexanoic acid **1a**. Therefore, it was expected that the mixed anhydride species **6b** (Fig. 3) would also exist in the analogous reaction with **1b**.

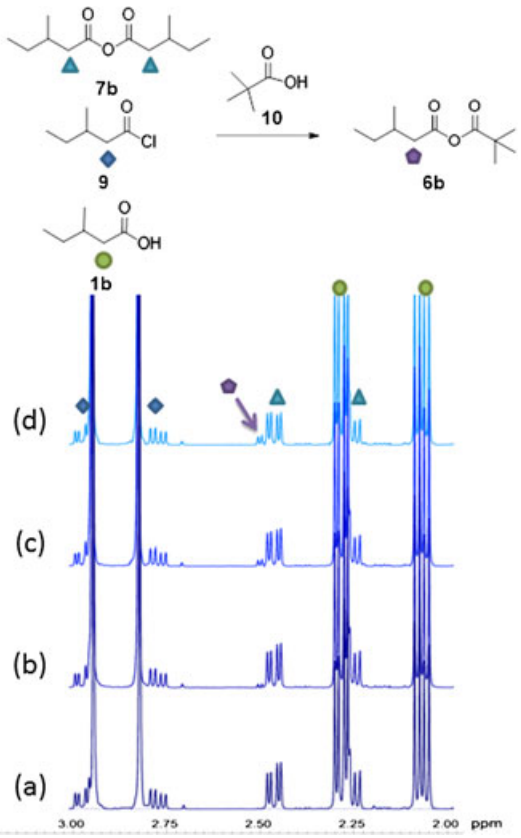


Figure 3. Expansion of the aliphatic region of the ^{1}H NMR spectra in acetonitrile-d_3: (a) 1 min, (b) 15 min, (c) 44 min and (d) 111 min after addition of pivalic acid **10** to the mixture from Figure 2c. Peaks corresponding to species **1b**, **7b** and **9** are highlighted.

The previous experiment demonstrated that **7b** was formed in the reaction mixture. If mixed anhydride **6b** was also present in the reaction mixture, it was expected that it would have similar methylene proton chemical shift to symmetrical anhydride. Therefore, an experiment was designed to generate a reference sample of **7b**. To achieve this, pivalic acid **10** was added to the sample of **1b**, **9** and **7b** that was generated in the previous experiment. This reaction generated a sample of the mixed anhydride **6b**, and resulting spectra are in Fig. 3. During the reaction, growth of signals at 2.25 and 2.46 ppm were observed, and 2D NMR spectroscopy experiments were used to confirm that these resonances were consistent with the mixed anhydride structure **6b**. Finally, the ^{1}H NMR spectrum was compared with that generated during the online NMR experiment, which correlated the mixed anhydride species **6b** with species **B** in the reaction profile shown in Fig. 1.

Figure 4 provides a summary of the diagnostic chemical shifts that were used to identify each species at 25 °C in acetonitrile-d_3. Many of these species have chemical shifts that are dependent upon reaction conditions, so the assignment displayed may vary slightly under actual reaction conditions.

A number of control experiments were also conducted to observe reactions between pivaloyl chloride and Meldrum's acid or *N*-methylimidazole. However, in both cases, no significant reaction was observed in the control experiments or in the complete reaction shown in Scheme 2.

Investigation into productive mechanistic pathways

After identification of each reactive intermediate, mechanistic investigations were performed to investigate which intermediates are on productive pathways. Because 3-methylpentanoyl chloride **9** was not previously identified in the mechanism, an experiment was designed to confirm if **9** reacts to form product **8b**. The chloride **9** was synthesized as previously described and added to Meldrum's acid **2** in the presence of excess 1-methylimidazole **4**. The reaction was monitored at 25 °C by ^{1}H NMR, and immediate growth of product **8b** occurred upon addition of **2** (Fig. 5). In this reaction, the anhydride species **6b** and **7b** could not form (which would have characteristic resonances at 2.25 and 2.46 ppm); therefore, acyl chloride **9** was the only productive intermediate to generate **8b**. This result indicated that **9** is present on a productive mechanistic pathway and does not merely exist as an intermediate to an anhydride.

In order to determine if the anhydride species **6b** and **7b** were also on a productive pathway, a mixture of the mixed anhydride **6b**, dimer anhydride **7b** and 3-methylpentanoic acid **1b** was

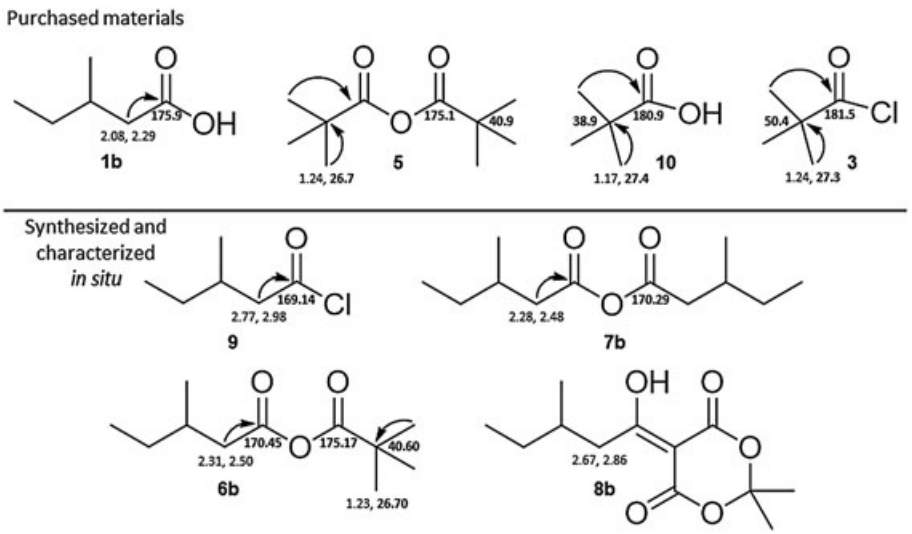


Figure 4. Summary of diagnostic chemical shifts in acetonitrile-d_3 at 25 °C for major species observed in this work. ^{1}H NMR assignments are in regular text and ^{13}C NMR assignments in bold. Curved arrows indicate ^{1}H–^{13}C HMBC correlations.

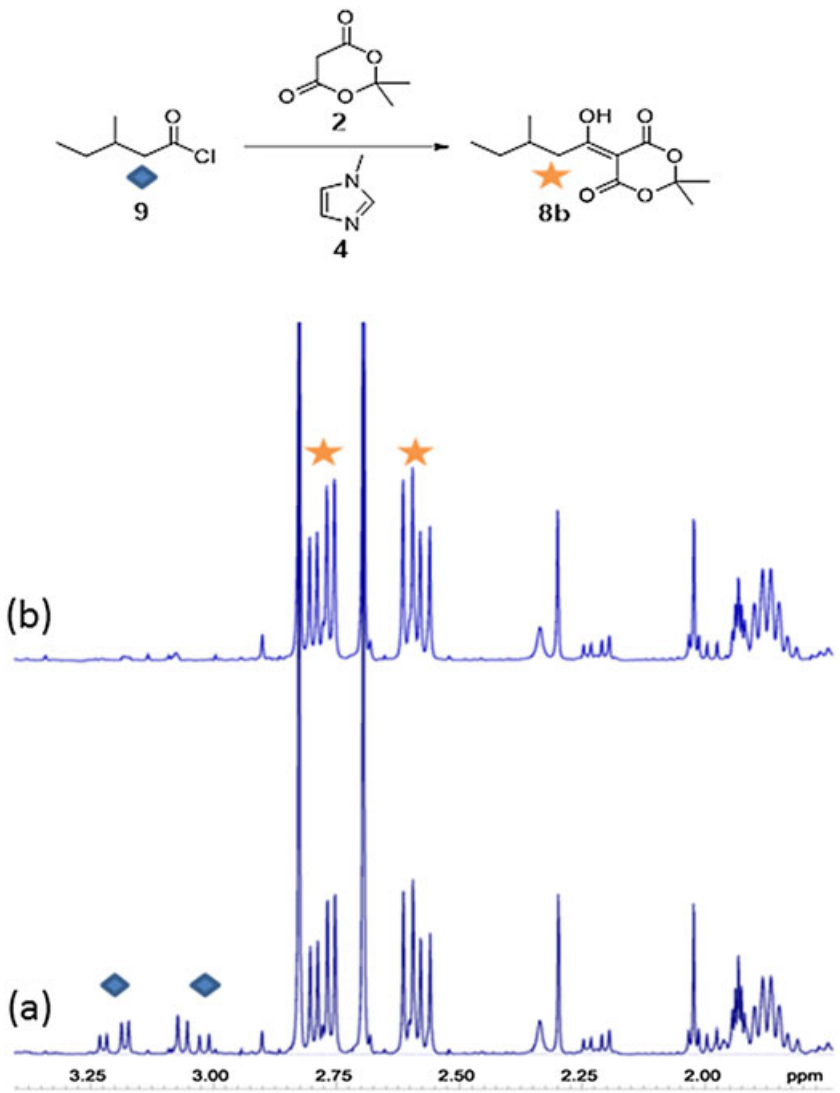


Figure 5. Expansion of the aliphatic region of the ^{1}H NMR spectra of the reaction between acyl chloride **9** and Meldrum's acid **2** in acetonitrile-d_3 after (a) 1 and (b) 13 min.

generated by reacting **1b** with pivalic anhydride **5** under basic conditions.[8] Meldrum's acid **2** was then added to this mixture, and growth of product was immediately observed (Fig. 6b). Under these reaction conditions, there was no chloride source present; therefore, **9** could not form. This experiment clearly demonstrated that the intermediate anhydride species **6b** and **7b** are also productive intermediates on the mechanistic pathway.

Clegg *et al.* indicated that pivalic anhydride **5** was an intermediate species for the reaction with **1a**. In that transformation, **5** could be have been generated by the reaction of pivalic acid **10** (a byproduct of the reaction) with **6a** or **3**. In order to observe if **5** was also an intermediate in the current study using starting material **1b**, the full reaction (Scheme 2) was monitored in an NMR tube at 0 °C with alternating proton and ^{1}H–^{13}C HMBC spectra. Pivalic anhydride **5** and pivaloyl chloride **3** display the same ^{1}H chemical shift for the *t*-butyl methyl, but their quaternary carbon chemical shifts are distinctive at 40.9 and 50.4 ppm. ^{1}H–^{13}C HMBC data recorded 15 min after reaction initiation showed ^{1}H–^{13}C HMBC correlations from the *t*-butyl methyl ^{1}H signals at 1.2 ppm to a quaternary ^{13}C signal at 40.9 ppm. This ^{13}C chemical shift correlation was consistent with those from a commercially available sample of **5**, indicating that **5** was formed during the reaction.

Acyl chloride formation

The source of the formation of the acyl chloride **9** was thought to be a reaction of chloride anion with anhydrides **6b** and **7b**. The only source of chlorine in the reaction mixture is pivaloyl chloride, which reacts with carboxylic acid species resulting in HCl, and formation of *N*-methylimidazole hydrochloride. In order to demonstrate that the acyl chloride is formed via reaction of chloride anion with the anhydrides **6b** and **7b**, a sample of these components was generated, and *N*-methyl imidazole hydrochloride was added. The proton NMR spectrum of this mixture showed the appearance of resonances that were previously demonstrated to be related to acyl chloride **9**. This result supported the hypothesis that the chloride anion could react with the anhydrides **6b** and **7b** to form the acyl chloride **9** as shown in Fig. 7.

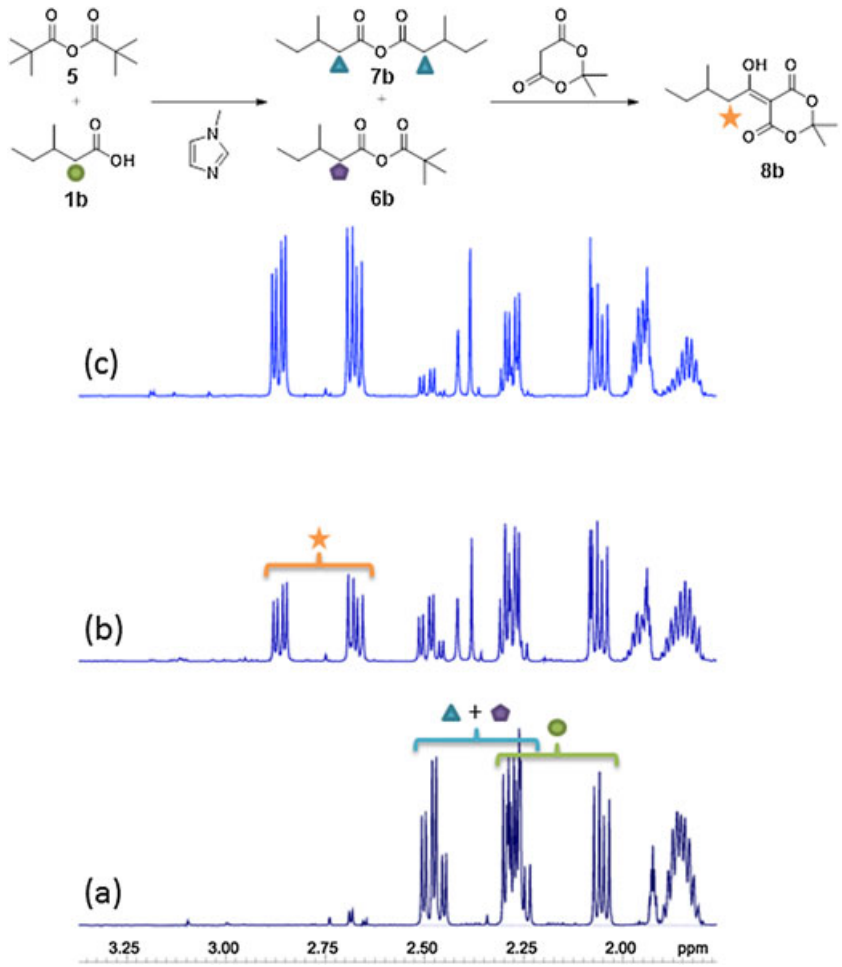


Figure 6. (a) Expansion of the aliphatic region of the ^{1}H NMR spectrum in acetonitrile-d_3 obtained after reacting **1b** with **5**; (b) 3.5 min and (c) 63 min after the addition of Meldrum's acid **2**.

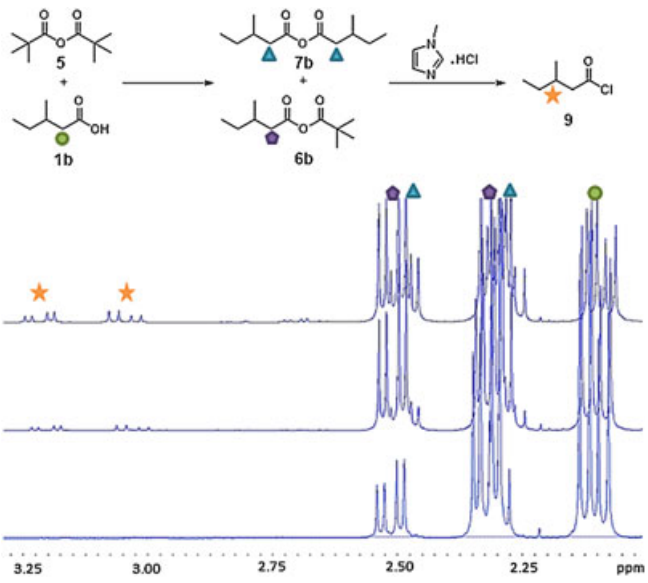
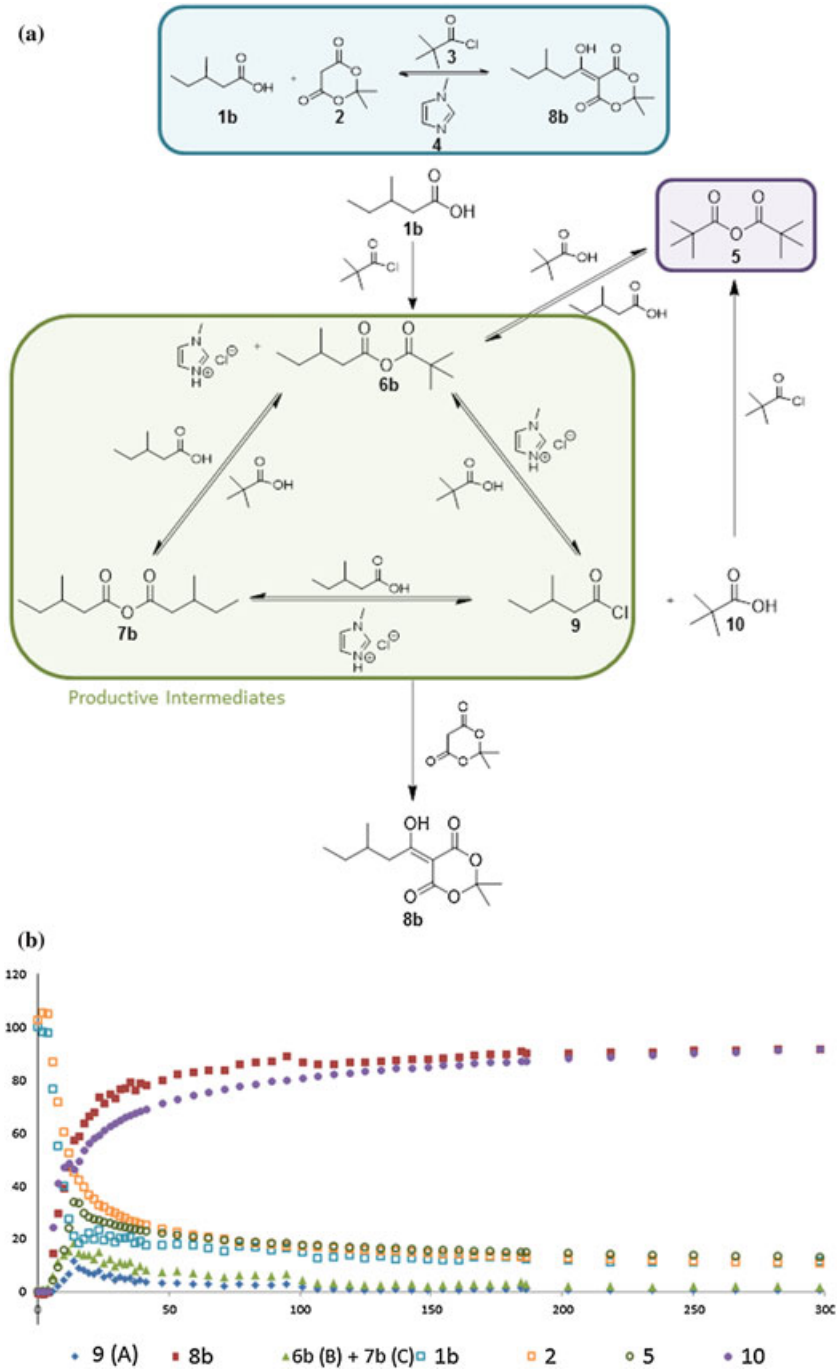


Figure 7. Formation of acyl chloride **9** by reaction of *N*-methylimidazole hydrochloride with anhydrides **6b** and **7b**.

Completion of reactive intermediate characterization revealed the full mechanism of this reaction and is summarized in Scheme 3. The starting material **1b** reacts with pivaloyl chloride **3** in order to produce the mixed anhydride species **6b** and the chloride salt of the base. Mixed anhydride **6b** can react with the chloride anion to produce the acyl chloride **9** and pivalic acid **10** or can also react with another equivalent of **1b** to produce the dimer anhydride **7b** and pivalic acid **10**. Mixed anhydride **6b** can also react with **10** to produce pivalic anhydride **5**. Reaction of pivalic acid **10** with pivalyl chloride **3** can also result in the formation of **5**. The mixed anhydride **6b**, dimer anhydride **7b** and acyl chloride **9** can each react with Meldrum's acid **2** to produce **8b**. A ^{1}H 2D ROESY experiment was performed on the reaction mixture to confirm the equilibrium between the intermediates involved. Exchange cross peaks were observed between acyl chloride **9**, mixed **6b** and symmetric anhydrides **7b**.

Conclusions

Online NMR was utilized to monitor the reaction of 3-methylpentanoic acid **1b** with Meldrum's acid **2,** and a detailed investigation into the reaction mechanism was undertaken. A combination of the online NMR experiment and a number of offline characterization experiments uncovered new details about this synthetic transformation, which had not been identified previously. Conclusive evidence is presented for the previously hypothesized dimer anhydride intermediate species. In addition, the presence of an acyl chloride intermediate species, 3-methylpentanoyl chloride **9**, was



Scheme 3. Summary of the key steps in the mechanistic pathway of the reaction.

discovered. The 3-methylpentanoic acid analogue **1b** is shown to have similar behavior under these reaction conditions as 3-methylhexanoic acid **1a**.

Online NMR is capable of providing a full reaction profile, which assisted in identification of new reactive intermediates in this synthetic transformation. This coupled with offline characterization of these intermediates yielded a detailed mechanistic picture of the synthetic step, building on the original work by Clegg *et al.* These results demonstrate how online NMR spectroscopy facilitates analysis of the constantly changing reaction system while also allowing standard unit operations such as reagent addition to be conducted effectively.

Experimental

General considerations

NMR spectra were acquired on either a Bruker 400 MHz AVANCE III NMR equipped with a BBFO probe or a Bruker 600 MHz AVANCE III NMR with a TXO probe. ^{1}H NMR spectra were processed with Bruker BioSpin, Billerica, MA, USA 3.2.pl6 and analyzed with Dynamics Center 2.2.4 (Bruker BioSpin, Rheinstetten, Germany).[9] Spectra were referenced to acetonitrile at δ_H 1.94 ppm. All reagents were purchased from Sigma-Aldrich Corp. St. Louis, MO, USA and used without further purification. Acetonitrile-d_3 was purchased from

Cambridge Isotope Laboratories, Inc. Tewksbury, MA, USA in 0.75-ml ampules and was used without further purification.

Online NMR spectroscopy

The online NMR system was used as previously described with the needle splitting valve removed.[7] The reaction vessel (Mettler-Toledo AutoChem Inc. Columbia, MD, USA), sample loop and spectrometer were all temperature controlled to 25 °C using a heating circulator (Julabo FP-50-HE) with Syltherm XLT temperature regulation fluid. The flow rate through the system was 3 ml/min regulated by a dual piston pump (Lab Alliance Prep 100, Scientific Systems, Inc. State College, PA, USA), and spectra were acquired on the flowing solution on a 400-MHz spectrometer. ^{1}H NMR spectra were acquired with four scans, 30° pulse angle and 10 s relaxation delay. 3-Methylpentanoic acid **1b** (3.5 ml, 28 mmol), 1-methylimidazole **4** (7.4 ml, 92 mmol) and Meldrum's acid **2** (4.5 g, 31 mmol) were added to 30 ml anhydrous acetonitrile in a reaction vessel under an atmosphere of N_2. Reagents were circulated through a system to achieve temperature (25 °C) and concentration equilibration. Pivaloyl chloride **3** (4.1 ml, 34 mmol) was added to reaction vessel over the course of 10 min via dosing syringe. ^{1}H NMR spectra were obtained on the flowing sample at 2 min intervals for the first 20 spectra, 6 min intervals until reaction time reached 185 min and then every 16 min until reaction completion was observed.

Offline NMR tube characterization

*Synthesis of 3-methylpentanoyl chloride **9***

3-Methylpentanoic acid **1b** (60 μl, 0.48 mmol) and three drops DMF were added to 0.75 ml acetonitrile-d_3. Oxalyl chloride (42 μl, 0.48 mmol) was added via a micropipette in two portions over 15 min at room temperature. ^{1}H, ^{13}C, HSQC and HMBC data were recorded for characterization of the reaction components.

*Synthesis of 3-methylpentanoic anhydride **7b***

To crude reaction mixture of synthesized 3-methylpentanoyl chloride **9** in an NMR tube, 3-methylpentanoic acid **1b** (50 μl, 0.40 mmol) and triethylamine (50 μl, 0.36 mmol) were added via a micropipette. ^{1}H, ^{13}C, HSQC and HMBC data were recorded for characterization of the reaction components.

*Synthesis of 3-methylpentanoic pivalic anhydride **6b***

To a crude reaction mixture of 3-methylpentanoic anhydride **6b**, 3-methylpentanoyl chloride **9** and 3-methylpentanoic acid **1b**, 5 mg pivalic acid **10** in an NMR tube at room temperature was added. The reaction was monitored at 25 °C with ^{1}H NMR.

*Synthesis of 3-methylpentanoic pivalic anhydride **6b** and 3-methylpentanoic anhydride **7b***

3-Methylpentanoic acid **1b** (62 μl, 0.5 mmol), pivalic anhydride **5** (102 μl, 0.5 mmol) and 1-methylimidazole **4** (131 μl, 1.65 mmol) were added to 0.75 ml acetonitrile-d_3 in an NMR tube at room temperature. ^{1}H, ^{13}C, HSQC and HMBC data were recorded for characterization of the reaction components.

*Reaction of 3-methylpentanoyl chloride **9** with Meldrum's acid **2** to produce 5-(1-hydroxy-3-methylpentylidene)-2,2-dimethyl-1,3-dioxane-4,6-dione **8b***

3-Methylpentanoyl chloride **9** (0.24 mmol) in 0.75 ml acetonitrile-d_3 was synthesized as described previously. A large excess of 1-methylimidazole **4** (2.5 mmol) was added to the solution of **9**. A bright yellow solid formed immediately upon addition and was filtered off. Meldrum's acid **2** was added to the filtrate via a micropipette at room temperature (36 mg, 0.24 mmol). The reaction was monitored at 25 °C with ^{1}H NMR.

*Reaction of 3-methylpentanoic pivalic anhydride **6b** and 3-methylpentanoic anhydride **7b** with Meldrum's acid **2** to produce 5-(1-hydroxy-3-methylpentylidene)-2,2-dimethyl-1,3-dioxane-4,6-dione **8b***

Meldrum's acid **2** (72 mg, 0.5 mmol) was added to the crude reaction mixture of 3-methylpentanoic acid **1b**, 3-methylpentanoic anhydride **7b** and 3-methylpentanoic pivalic anhydride **6b** in an NMR tube at room temperature. The reaction was monitored at 25 °C with ^{1}H NMR.

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