NMR Spectroscopy

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Real-Time Monitoring of Organic Reactions with Two-Dimensional Ultrafast TOCSY NMR Spectroscopy**

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Multidimensional nuclear magnetic resonance plays a number of essential roles in present day spectroscopy. It is also an integral part of the image formation protocol in magnetic resonance imaging (MRI).[1] Traditional two-dimensional experiments are intrinsically time consuming, because many t_1 increments have to be acquired to obtain two-dimensional spectra with adequate digital resolution in the indirect dimension.[2] Proposals for accelerating multidimensional NMR spectroscopy include non-Fourier transform schemes,[3] the acquisition of multiple NMR spectra in a single experiment,[4] and "single scan" multidimensional NMR spectroscopy, also called ultrafast NMR spectroscopy (UF NMR), have been introduced. The latter methodology was inspired by echo planar imaging (EPI)[5] and was developed by Frydman et al.[6] It permits the collection of complete multidimensional NMR data sets within a single continuous acquisition. This new methodology is compatible with existing standard (TOCSY, HSQC, MRI) and recently described combined multidimensional pulse sequences,[7] and it can be implemented with conventional hardware. This attractive feature enables ultrafast NMR to examine dynamic processes, that is, organic reactions and their mechanisms as they happen in real time.[8]

Figure 1 shows the schematic of the two-dimensional UF-TOCSY sequence used.[9] The sequence uses a continuous spatial encoding[10] which was implemented by pairs of RF pulses which excite/store spins over the full length, L, of the sample. The sample was swept over intervals $t_1^{max}/2$ whereas $\pm G_e$ external gradients were applied. The offsets of such pulses were thus chirped over a $\pm\gamma G_e L/2$ span, and their amplitudes calibrated as effective $\pi/2$ nutations by setting γB_1 as a function of $0.25[(2\gamma G_e L/t_1^{max})]^{1/2}$.

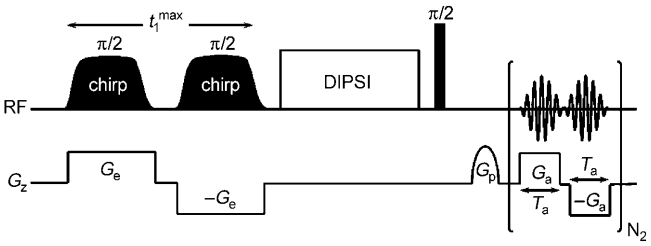

Figure 1. The ultrafast two-dimensional TOCSY used to carry out real-time characterizations. DIPSI = decoupling in the presence of scalar interactions.

We focused our attention on one-pot syntheses of pyrimidines and similar heterocyclic compounds, which we had previously developed.[11] Pyrimidines are an important class of compounds which includes numerous natural, pharmaceutical, and functional materials.[12] Elegant new procedures have been described[13] and revealed decisive information on the mechanistic details of the reaction between carbonyl compounds and strong electrophiles such as trifluoromethanesulfonic acid anhydride (Tf_2O).[11,14] In spite of the importance of these reactions, no spectroscopic confirmation of the postulated intermediates or kinetic data on the reaction have been reported.

Using a medium field 500 MHz spectrometer, we have now applied UF NMR methodology to monitor the reaction between aliphatic ketones and Tf_2O in the presence of nitriles. We have determined how the signals of the starting and final products evolve as they happen, in real time and attempted to detect the presence of intermediates. We chose a symmetric aliphatic ketone, 3-pentanone (**1**), as the model compound to react with a two-fold amount of Tf_2O in [D_3]acetonitrile, which served as both a co-reactant and solvent.

Scheme 1 shows the previously proposed reaction mechanism,[14] which begins with the electrophilic attack of triflic anhydride onto the ketone (**1**) to form the short-lived (trifluoromethanesulfonyl)carbenium ion **2**. Cation **2** can undergo 1) rapid elimination of a proton leading to a mixture of *Z*/*E* vinyl triflates **3**, or 2) trap two molecules of acetonitrile in quick succession, and then undergo elimination of triflic acid and cyclization to finally lead to pyrimidine **7**. The formation of pyrimidine in high yield (> 95 %) accompanied by a small amount (5 % <) of vinyl triflate indicates[11] that the efficient nucleophilic trapping of the cationic species **2** occurs faster than the loss of a proton. The trapping pathway leads to the formation of the triflyloxy nitrilium salt intermediate **4**, which then captures another molecule of acetonitrile to give the intermediate **5**. The elimination of

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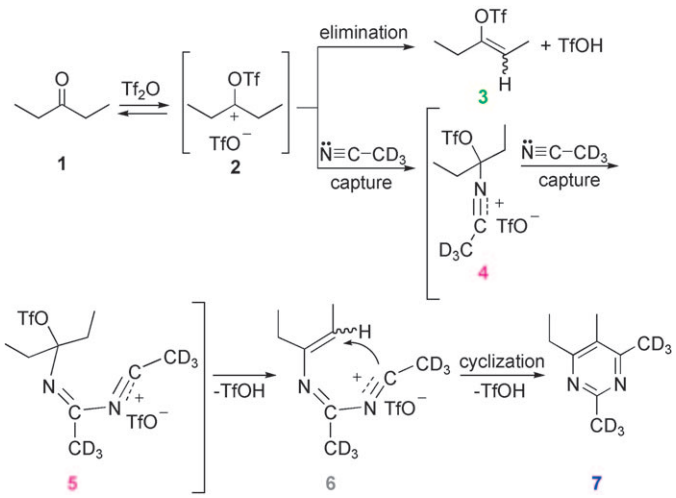


Scheme 1. The reaction between ketones and triflic anhydride in the presence of nitriles as nucleophiles.

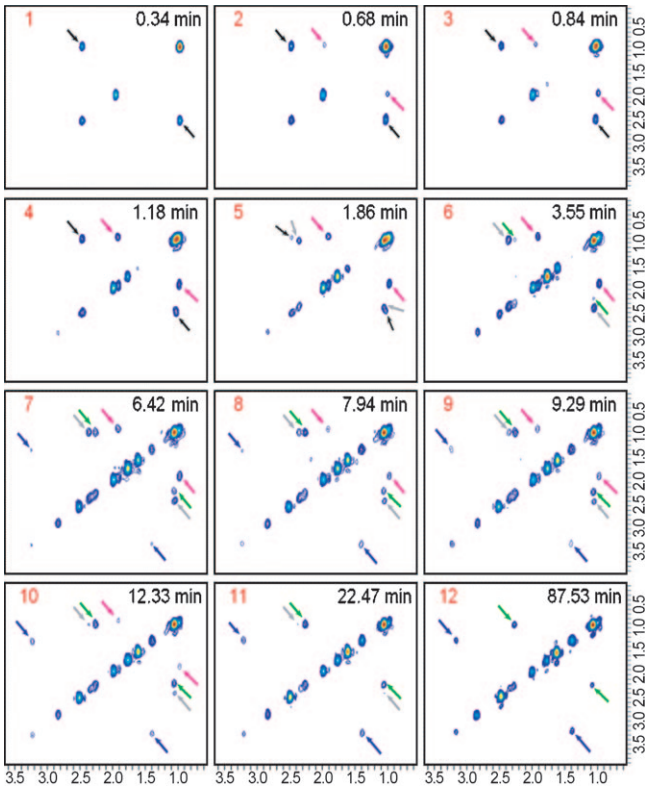


Figure 2. A series of two-dimensional TOCSY NMR spectra taken at different times throughout the reaction (scale is in ppm). 525 TOCSY experiments were acquired. Data were collected at 37 °C at approximately every 10.0 s following the sequence depicted in Figure 1. Time points are shown within each window..

triflic acid from **5** takes place easily since the triflate anion is a formidable leaving group,[15] leading to the *Z*/*E* olefinic nitrilium intermediate **6**. Data about the species **5** have not been described although nitrilium salts are present in different reactions having electrophilic reagents, and should be relatively stable since the positive charge can be delocalized onto either the nitrilium nitrogen atom or onto the nitrilium carbon atom.[16] Both *Z*- and *E*-**6** lead, after rapid cyclization and loss of triflic acid, to the same pyrimidine **7** (95 % yield).

We applied the UF TOCSY sequence (Figure 1) to the study of this reaction and recorded 525 two-dimensional TOCSY experiments. Values of various parameters controlling the acquisition and excitation gradients were optimized, and the excitation pulses were chosen to achieve optimum resolution without appreciable loss of spectral width.

The aliphatic range $\delta = 0.00$–3.70 ppm was examined and changing concentrations of cross peaks representing the coupled methylene and methyl hydrogen atom signals of the ethyl groups from the species present were noted. A mixing time of 20 milliseconds produces COSY cross-peaks. Higher values permit the detection of TOCSY interactions, but in this case led to overcrowded spectra. The species were assigned structures on the basis of their substituents. Species **1**, **3**, **4**, **5**, **6**, and **7** were monitored and their appearance and disappearance were determined in real time, thereby revealing important information about the mechanism of the reaction.

The addition of the reactants employed a simple fast mixing device (see the Experimental Section). We estimate that this assembly has a kinetic dead time of 1–2 seconds, which is sufficiently short to permit the detection of certain transient intermediates and determine kinetics by using UF TOCSY.

Figure 2 illustrates a series of 12 representative two-dimensional TOCSY spectra which were recorded in succession and numbered 1–12 (referred to as TOCSY-1, etc.) Cross-peaks assigned to starting and final products were confirmed using standard one-dimensional and two-dimensional spectra. TOCSY-1 corresponds to a solution of the ketone **1** in [D_3]acetonitrile. Cross-peaks from the ethyl group ($\delta = 2.48$–1.04 ppm, black arrows) are clearly observed. The spectra in TOCSY-2–TOCSY-12 reflect the reaction mixture after the addition of the solution of ketone **1** in [D_3]acetonitrile to a solution of Tf_2O in [D_3]acetonitrile. In TOCSY-2, 0.68 minutes after the addition, new cross-peaks appear at lower frequencies ($\delta = 1.97$–1.00 ppm, magenta arrows). These peaks intensify until TOCSY-4 (1.18 min) then decrease and fall below the recording limit after TOCSY-10. This variation in intensity is more clearly shown in Figure 3, which displays the intensities of the cross peaks as a function of time. Clearly the cross-peaks represent an intermediate which is formed directly from ketone **1**. The intensity of the cross-peaks from ketone **1** decrease exponentially and are absent after TOCSY-5 (1.86 min; also see Figure 3). The formation of a new intermediate **6** (2.34–1.06 ppm, grey arrows) is initially detected in TOCSY-5, and one of its cross-peaks partially overlap with one from ketone **1** because of insufficient resolution along the indirect dimension.[17] The intensity of the peak increases rapidly until TOCSY-7, then decreases (TOCSY-8–TOCSY-11) and is no longer recorded after TOCSY-12.

The formation of the *Z*/*E* vinyl triflate side products **3** ($\delta = 2.36$–1.08 ppm, green arrows) is initially detected in TOCSY-6 and is observed up to the end of the sequence. New cross-peaks caused by the ethyl group of the final pyrimidine **7** are initially detected in TOCSY-7 ($\delta = 3.15$–1.38 ppm, blue

arrows). The intensities of the cross-peaks for 7 increase exponentially and level off toward the end of the sequence. The positions of the signals from the *Z*/*E* vinyl triflates 3 and the pyrimidine 7 were confirmed through standard one-dimensional and two-dimensional experiments (see the Supporting Information).

We assigned the new correlations (magenta arrows) to the nitrilium salt intermediates 4 and 5 (Scheme 1). Their calculated chemical shifts (ACD/Labs 8.00 Release) are in agreement with the new signals observed. The intermediate nitrilum salts 4 and 5 appear as a mixture at an early stage of the reaction (TOCSY-2, 0.68 min), prior to the appearance of the signals from the *Z*/*E* vinyl triflates 3 (TOCSY-6, 3.55 min), confirming that the short lived species **2** is captured faster by Tf_2O than it loses a proton. Intermediate 5 easily eliminates TfOH to afford the olefinic nitrilium salt intermediate 6 (TOCSY-5, 1.86 min). Probably as a result of their low intensity, no allylic correlations could be detected. The presence of the double bond causes the cross-peaks from the ethyl group of the intermediate 6 to appear at higher field than those in intermediate 5. These cross-peaks persist until TOCSY-11. An animation of this process was made using the first 211 TOCSY experiments (see the Supporting Information).

Figure 3 shows the intensities versus time of the various species that were as part of the reaction mixture. The intermediate characteristics of 4, 5, and 6 are shown with their rise and fall during the course of the reaction. The $t_{1/2}$ lifetimes of the starting ketone **1**, intermediates 4, 5, and 6 were obtained by fitting the data points to the equation $I_{(\tau)} = I_0\exp(-\tau/t_{1/2}) + I_{-}$. Values shown in Figure 3 gave reaction rate measurements which permit evaluation of the reactive behavior of the intermediates. The points chosen were taken from measurements starting at 0.0, 1.2, and 3.2 minutes respectively.

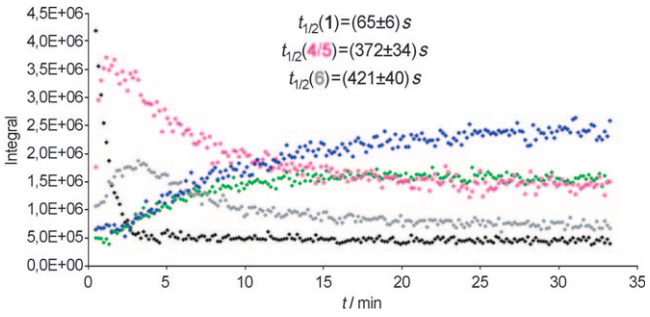
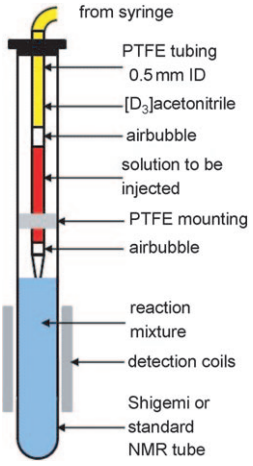


Figure 3. The averaged integrated peak intensity as a function of time for reactant **1** (◆), intermediates 4, 5 (◆), and 6 (◆), and final products 3 (◆) and 7 (◆).

In conclusion, the reaction between a simple ketone, 3-pentanone, and triflic anhydride in the presence of [D_3]acetonitrile was monitored by two-dimensional UF TOCSY using a standard 500 MHz spectrometer and a 5 mm NMR tube. The evolution of the reactants, presence of intermediates, and generation of reaction products were observed. These results represent another example of the possibilities made accessible by the UF NMR methodology. Real-time monitoring of the multistep reaction described above revealed important data about its mechanistic and kinetic aspects. Additional studies about new applications of the ultrafast methodology are in progress in our laboratory.

Experimental Section

A solution of Tf_2O (29.6 mg; 150 mM) in CD_3CN (0.5 mL) was prepared and added to a 5 mm NMR tube. A fast mixing device was devised and consisted of a long Teflon injection tube which connected a syringe with a Luer-lock tip to the reaction mixture inside a simple 5 mm NMR tube (see diagram). The NMR tube was fitted with a cap having a hole and a bearing to minimize oscillations of the injection



tube. In the fully loaded position, the injection tube contained, in order from the bottom tip upward: an air bubble (ca. 10 μL; about 1–2 cm length), the solution to be injected [3-pentanone (6.03 mg; 100 mM) of in CD_3CN (2.0 mL)], another air-bubble, about (20 μL; about 3–4 cm in the injection tube). The upper part of the injection tube was filled with CD_3CN to efficiently propagate the pressure throughout the injection tube. The bottom end of the injection tube was 1–2 mm inside the solution (see figure). The plastic cap was adjusted to maintain the injector at the correct height inside the NMR tube. The vertical position of the NMR tube was adjusted with the tube spinner and best results were achieved when the bottom of the NMR tube was approximately 10 mm below the lower end of the detection coil. Once the NMR tube with the spinner and the injection tube was fully assembled and placed into the detection coil zone, the bottom tip of the injection tube was well above the detection coil. Standard NMR adjustments were carried out before starting the TOCSY experiments. Acquisition of TOCSY experiments were started 20 s (2 scans) before the injection of the 3-pentanone solution and scans were recorded every 10 s. A total of 525 TOCSY scans were recorded. The acquisition parameters were: bandwidth of chirp pulse: 60 kHz; $G_e = 8\ G\,cm^{-1}$; $t_1^{max}/2 = 10$ ms; $G_a = 20\ G\,cm^{-1}$; $T_a = 0.246$ ms; $N_2 = 64$; gradient switching time = 40 μs. These parameters correspond to a spectral window of SW1 = 3.63 ppm and SW2 = 3.50 ppm. A sinusoidal purge gradient of 16 $G\,cm^{-1}$ during 200 μs was applied before acquisition. The data were sampled every 1 μs. Time used for the DIPSI sequence was 20 ms. For each experiment a suitable shearing was carried out and data were zero filled before the T2 Fourier transformation. Spectra were represented in magnitude

mode. To carry out such calculations a variety of Matlab 7.3.0 (Math Works Inc.) programs were developed.

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