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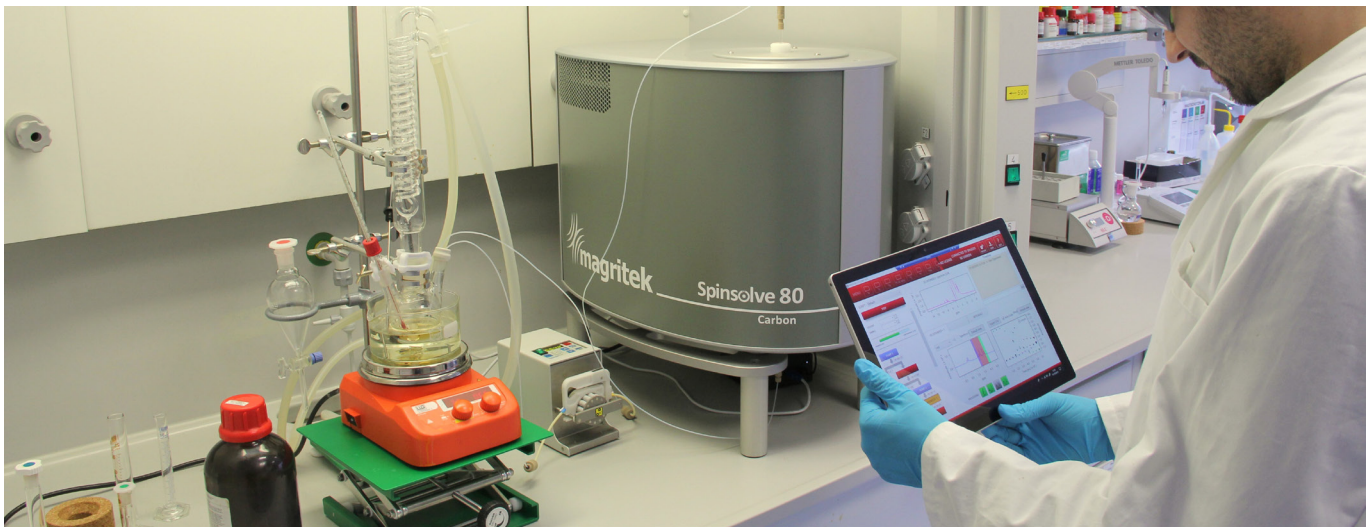
On-line reaction monitoring of an S_NAr reaction by ^1H and ^{19}F NMR



Fluorine containing scaffolds and building blocks play a remarkable role within drug discovery in medicinal chemistry for the synthesis of Active Pharmaceutical Ingredients (API). The fluorine atom holds some key advantages in terms of pharmacokinetic and physicochemical properties. Some of the major advantages are the improved metabolic stability and the enhanced membrane permeation that allow for a significantly higher bioavailability of fluorine-containing drugs [1].

In terms of NMR spectroscopy, the analysis of the ^{19}F nucleus is very similar to collecting ^1H-NMR data. ^{19}F has spin ½, a natural abundance of 100%, and its resonance frequency is very similar to ^1H (e.g. 60 MHz ^1H vs. 56 MHz ^{19}F) [2]. In contrast to the ^1H nucleus, it has additional p-orbitals which result in higher chemical shifts compared to the shifts of proton signals. It also has lower longitudinal relaxation times (T_1), which allows for faster scan times. All these properties make fluorine an ideal nucleus for acquiring spectroscopic data and follow its transformation over the course of a chemical reaction.

On-line reaction monitoring by NMR has proven to be a valuable tool in process control, as it allows for a deep insight into mechanisms and kinetics of chemical reactions at laboratory, as well as pilot-scale. In previous Application Notes, we have demonstrated the benefits of using the Spinsolve™ Reaction Monitoring Kit to follow the progress of different reactions by ^1H-NMR spectroscopy. In this application note, we show how the course of an S_NAr reaction can be monitored on-line by acquiring both ^1H- and ^{19}F-NMR spectra sequentially in a continuous mode. For these particular experiments we employed our Spinsolve™ 60 MHz HF ULTRA system, but all Spinsolve™ models come with capabilities to acquire ^1H- and ^{19}F-NMR spectra without retuning or recalibration.

The possibility to switch from ^{1}H to ^{19}F allows us to enhance the analytical power of our spectrometers to obtain additional process information. To perform kinetic studies where both ^{1}H- and ^{19}F-NMR spectra are acquired in an interleaved way, the Spinsolve™ Reaction Monitoring software module was utilized. Besides the ease of use, this module offers high flexibility when defining the monitoring mode, setting the parameters of the different measurement protocols, and synchronizing the pump control according to the time-resolution and sensitivity required.
Figure 1 shows a print screen of the new reaction monitoring software protocol that enables full acquisition of ^{1}H and ^{19}F spectra. The software module also provides on-the-fly processing and evaluation capabilities, a flexible adjustment of measurement parameters during the course of the reaction, and an automated output of the data in several data formats (e.g. csv-file).

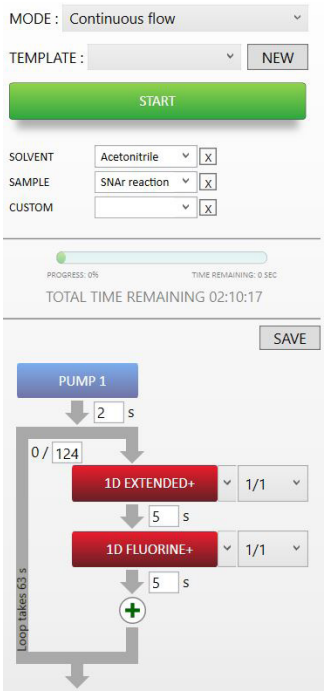
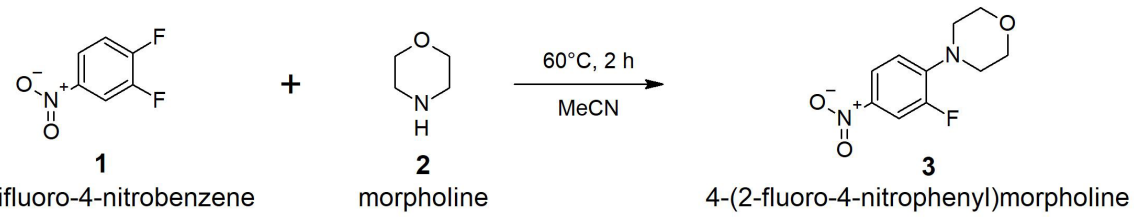


Fig. 1: Print screen of the new Spinsolve™ reaction monitoring software protocol programmed for the continuous acquisition of ^{1}H- and ^{19}F-NMR spectra with automated pump control.

General set-up and conditions

The reaction investigated in this kinetic study is the S_NAr reaction of 1,2-difluoro-4-nitrobenzene (1) and morpholine (2) to yield the desired 4-(2-fluoro-4-nitrophenyl)-morpholine (3). The selective formation of (3) is feasible due to the mesomeric effect within the aromatic ring system of the nitrobenzene (1) implied by the nitro group. The electron withdrawing nitro group activates the ortho and para position by reducing its electron density. Therefore, the fluorine moiety in para position in respect of the nitro group is more prone to a nucleophilic attack and a substitution with a suitable nucleophile than the neighbouring fluorine atom. So, in this case we have a substrate controlled regioselectivity (Scheme 1).



1 1,2-difluoro-4-nitrobenzene + **2** morpholine → **3** 4-(2-fluoro-4-nitrophenyl)morpholine

Scheme 1: S_NAr reaction for the synthesis of 4-(2-fluoro-4-nitrophenyl)morpholine (3).

Background and practical significance

This particular reaction is the starting point of a multi-stage synthesis for the class of 3-aryl-2-oxazolidinones, which represent a potential new type of antibiotic agents [4]. Due to the excessive use of antibiotic substances in several application fields within recent decades, multi-resistant strains of bacteria have become a major problem for health care practitioners. Many pathogens acquire resistance even to formerly known “last resort” compounds like vancomycin. The most famous of these bacterial strains is *Staphylococcus aurea*, which is better known as MRSA, and can be found in almost every medical facility worldwide. Therefore, the development of new highly effective antibiotic agents is one of the highest priority tasks of the pharmaceutical industry today.

The reaction was studied in batch and conducted in a three-necked round-bottom flask with a total volume of 150 mL. The flow-system was set up by using the Spinsolve™ Reaction Monitoring Kit 2, which includes a glass-flow cell and a peristaltic pump connected in a closed loop with the reaction vessel. The reaction mixture was circulated continuously through the instrument with a flow rate of 0.8 mL/min (Figure 2). 1,2-difluoro-4-nitrobenzene (1) (3.979 g, 25 mmol, 1 eq.) was added dropwise to a solution of morpholine (2) (4.361 g, 50 mmol, 2 eq.) in 18 mL of protonated acetonitrile at room temperature. The reaction mixture was heated to 60°C after the addition was finished. After two hours of heating, the mixture was stirred overnight at room temperature, resulting in a yellow colored liquid containing a white precipitate.

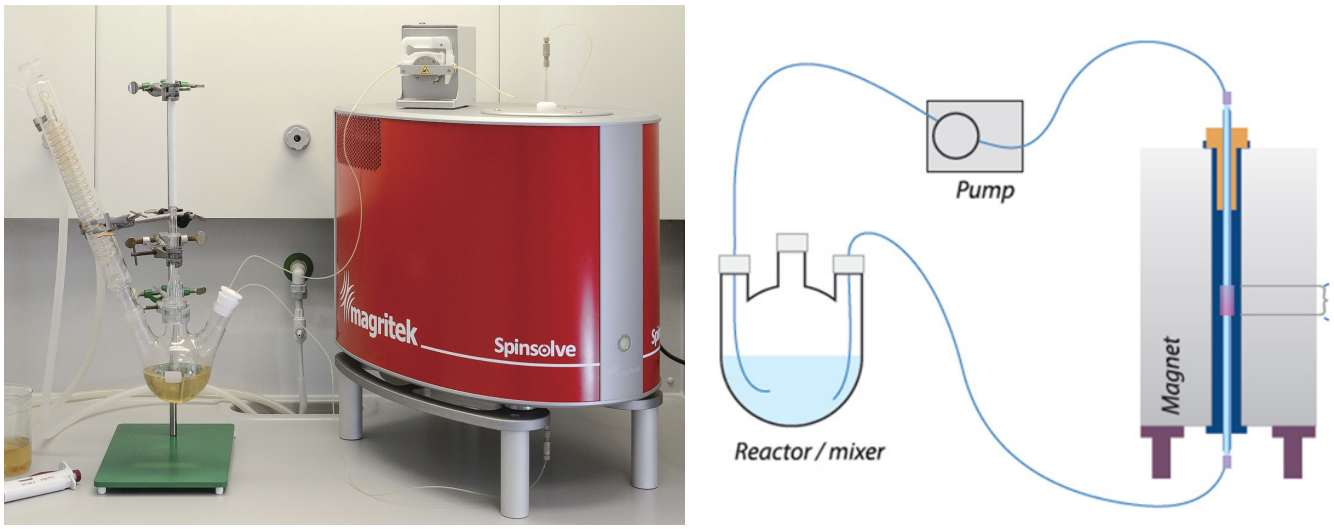


Figure 2: Set-up of the S_NAr reaction in a fume hood in real life (left) and as a scheme (right).

Results of Reaction Monitoring study

In order to identify the signals that should be monitored to follow the kinetics, we measured NMR spectra of the starting materials and of the reaction mixture at the given concentrations in acetonitrile to identify the regions of interest in the spectra that could be integrated to quantify the different components of the reaction. Figure 3 shows a stack plot of the ^{1}H-NMR data of the starting materials and the reaction mixture. The NMR spectrum of 1,2-difluoro-4-nitrobenzene (1) is shown at the bottom of the stack plot. The typical NMR signals in the aromatic region are visible together with the signal of acetonitrile at about 2.0 ppm (the two carbon satellites of acetonitrile at about 0.8 and 3.0 ppm are marked in blue).

It is very common in reaction monitoring experiments, where protonated solvents are typically used, to observe the carbon satellites of the solvent with a size comparable, or even larger, than the peaks of the products of interest. The morpholine scaffold (2) only shows peaks in the aliphatic region. There are three signals in total. The first signal appears at about 1.7 ppm, which belongs to the NH-moiety. The two peaks of the symmetrical eight ring protons appear at 2.6 and 3.5 ppm. The ^{1}H-NMR spectrum of the reaction mixture can be observed on the top inset. The peaks of the solvent and the morpholine are perfectly aligned with the peaks in the separately recorded NMR spectra of the starting materials. We identified three additional signals corresponding to (3), which do not overlap either with the solvent peak nor with its satellites. Two peaks appear at 3.2 and 3.6 ppm and one is visible at about 7.0 ppm (marked in red). For this particular experiment there was no need for either suppressing the solvent signal nor to use a carbon decoupling protocol to eliminate the carbon satellites, as they are not overlapping with the peaks of interest. The NMR spectra shown in Figure 3 were acquired with a single scan. This short measurement time still delivers a high signal-to-noise-ratio.

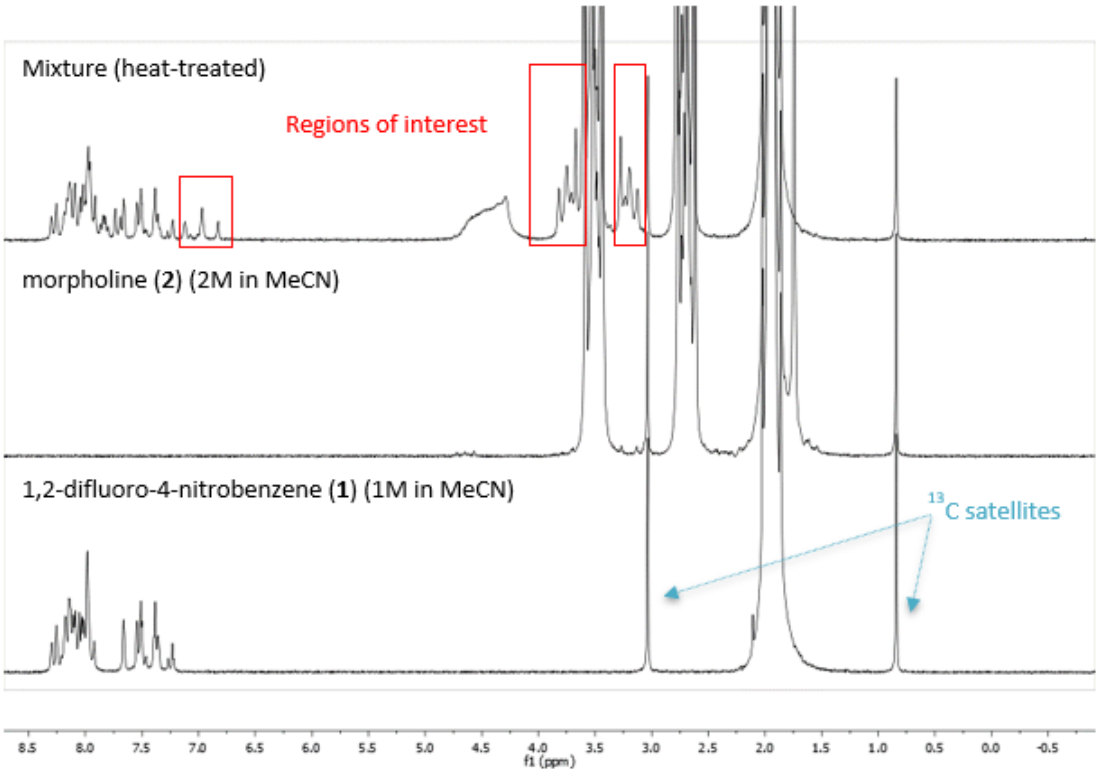


Figure 3: ^{1}H-NMR spectra (stacked) of the starting materials and the reaction mixture all recorded separately.

The reaction was monitored by acquiring a total of 124 spectra for each nucleus over a time period of about 130 minutes (see Fig. 4 left stack plot). The peaks of interest in the ^{1}H-NMR spectra are the ones in the regions identified before at 3.2, 3.6, and 7.0 ppm. They were integrated as described in Figure 3. What attracts the attention in the ^{1}H-NMR spectra, is the peak shifting from 1.7 ppm all the way to 9.6 ppm. This peak belongs to the NH-moiety of the morpholine (2) (orange). Due to pH changes during the reaction, this labile dynamic proton is moving from one side of the spectra to the other. The stack plot on the right side of Fig. 4 shows the ^{19}F spectra collected in an interleaved way during the reaction. We can identify two peaks at -134 and -127 ppm belonging to the 1,2-difluoro-4-nitrobenzene (1) (red and blue). These peaks decrease over time while a peak at -119 ppm corresponding to product 3 appears (orange). As the reaction progresses, hydrofluoric acid is formed as a leaving group which contributes significantly to the pH variation during the reaction. The NMR peak for hydrofluoric acid appears at about -145 ppm in the ^{19}F-NMR spectra and slightly drifts towards higher ppm values to finally stay at about -139 ppm (green). Due to the large chemical shift range in ^{19}F-NMR, the chance of overlapping peaks is very low as it can be seen in this example. Moreover, a white solid precipitated out of the reaction mixture which we assume to be the hydrofluoric salt of the morpholine (2) employed in excess during this process. Despite the obstacles introduced by the drifting peak and the precipitation, we were able to identify the signals of the different components in both the ^{1}H-NMR as well as the ^{19}F-NMR spectra. By integrating the corresponding regions in the spectra we succesfully followed the course of the reaction (Figure 4).
Figure 5 shows the different concentrations obtained from the ^{1}H- and ^{19}F-NMR spectra plotted as a function of time. We can clearly see the formation of product (3), and confirm that about half of the morpholine (2) is consumed (orange region, left). For product (3) we have marked the integral regions of interest in blue, green, and red. The blue integral region at 7.0 ppm correspond to the single proton at position 12 of the product 3. It reaches a steady state at the absolute integral value of 100. The green and red integral regions display the aliphatic ring protons of the product 3 and appear at 3.2 and 3.6 ppm, respectively. The green region belongs to the four ring protons at positions 3 and 5 while the red region belongs to the four protons at positions 2 and 6.

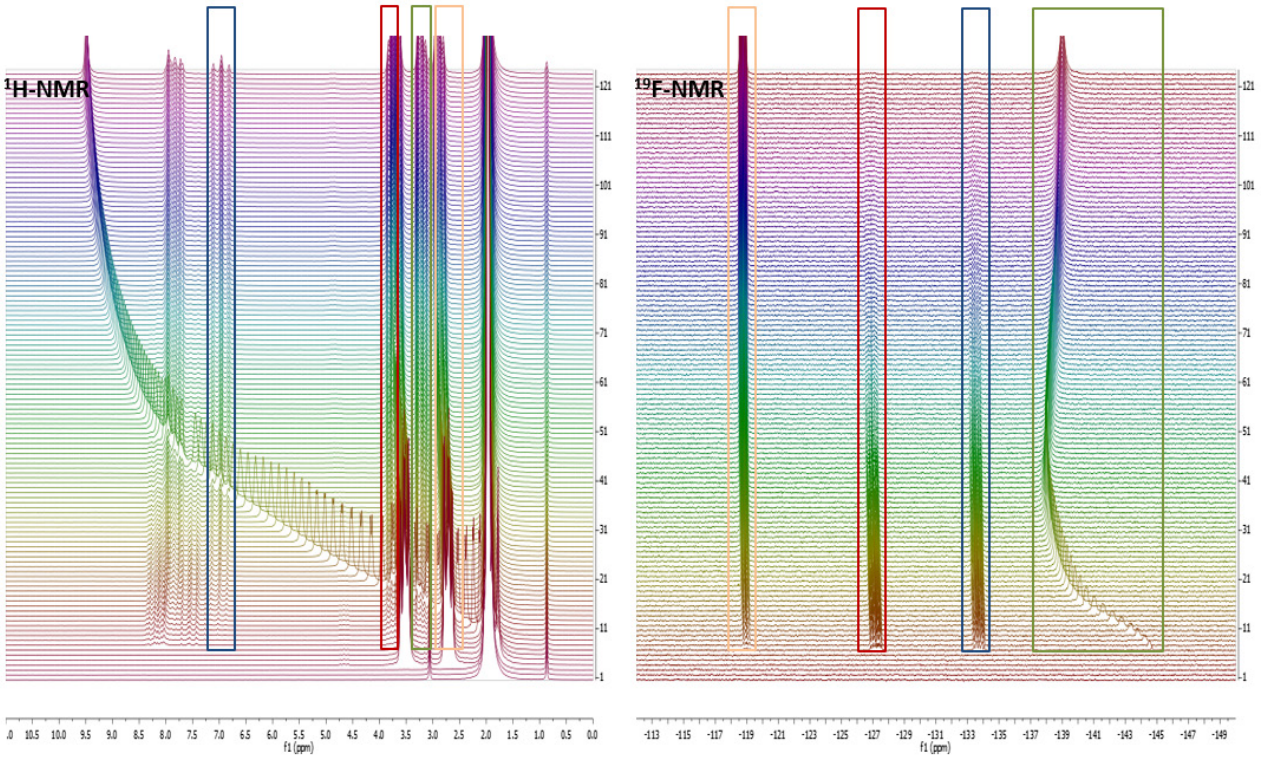


Figure 4: ¹H-NMR spectra (stacked left side) and ¹⁹F-NMR spectra (stacked right side) of the kinetic study with an overall spectra number of 124 for each nucleus recorded sequentially.

The aliphatic protons have, each, an absolute integral value of about 400, which agrees quite well with the four protons in each chemical group. As it can be noted, the trend of the reaction can be followed by monitoring any of the signals of product 3, even though the morpholine (2) NH-peak overlapped at certain times with the peaks of interest. Due to a slight drift of the morpholine (2) signal at 3.5 ppm towards the red region, these two regions are not perfectly separated from each other, which is the reason why the data points for the red region do not perfectly match with the green ones (Figure 5).

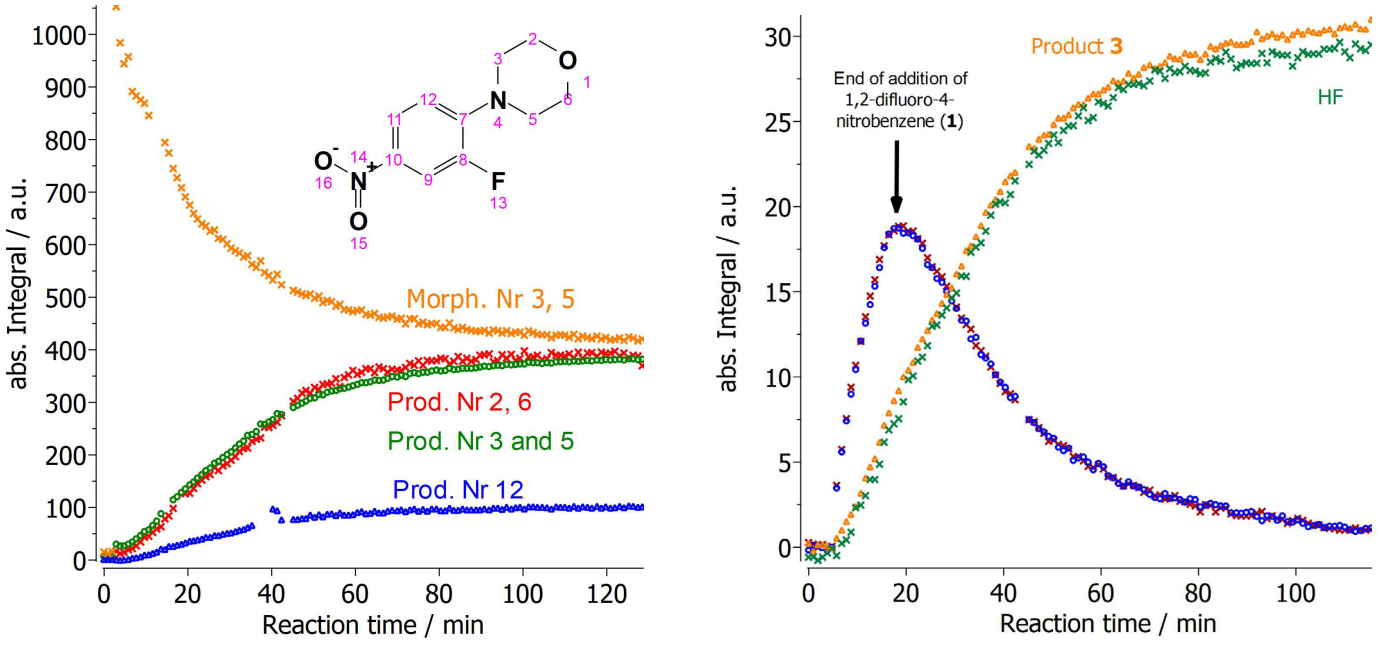


Figure 5: Trends of ¹H- and ¹⁹F-NMR spectra over reaction time based on integrated regions of interest for reactants morpholine (2) and 1,2-difluoro-4-nitrobenzene (1) and three product 3 specific ranges (left) as well as HF and the fluorine product 3 peak (right).

The integrals of the signals at -127 and -134 ppm from the ^{19}F-NMR spectra (right stack plot in Fig. 4) can be used to follow the addition and consumption of the reactant 1,2-difluoro-4-nitrobenzene (1). It can be clearly observed how the signal amplitudes increases during the first 20 minutes, as 1 is added to the mixture, and afterwards decreases exponentially once the addition is completed (marked in red and blue). On the other hand, the orange integral region at -119 ppm belongs to the fluorine signal of product (3). This signal increases during the time of the reaction showing the same trend as for the signal at -145 ppm, which we assume belongs to the hydrofluoric acid produced during the process as a side product (marked in green). Due to the large chemical shift range of the fluorine nucleus, the chance of having overlapping peaks is very low. Even the HF signal drifting from -145 ppm to -139 ppm introduces no problem in terms of peaks overlapping. Furthermore, there is no signal of the solvent nor morpholine (2) visible in the ^{19}F-NMR which makes the fluorine kinetic study very easy to process and evaluate. These results demonstrate that the possibility to measure ^{19}F-NMR spectra interleaved with the ^{1}H spectra without the need of any hardware adjustment enhances the kinetic information that can be obtained during on-line monitoring experiments conducted with a Spinsolve™ benchtop NMR system.

References

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[3] Application Note 13 - Online Monitoring of the N-Acetylation Reaction of L-Phenylalanine

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