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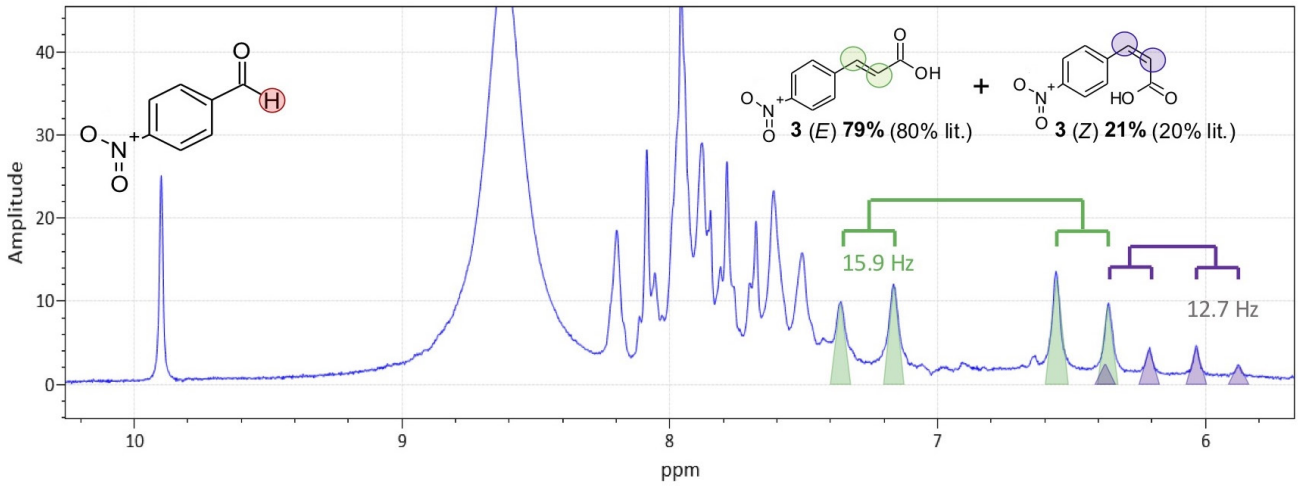
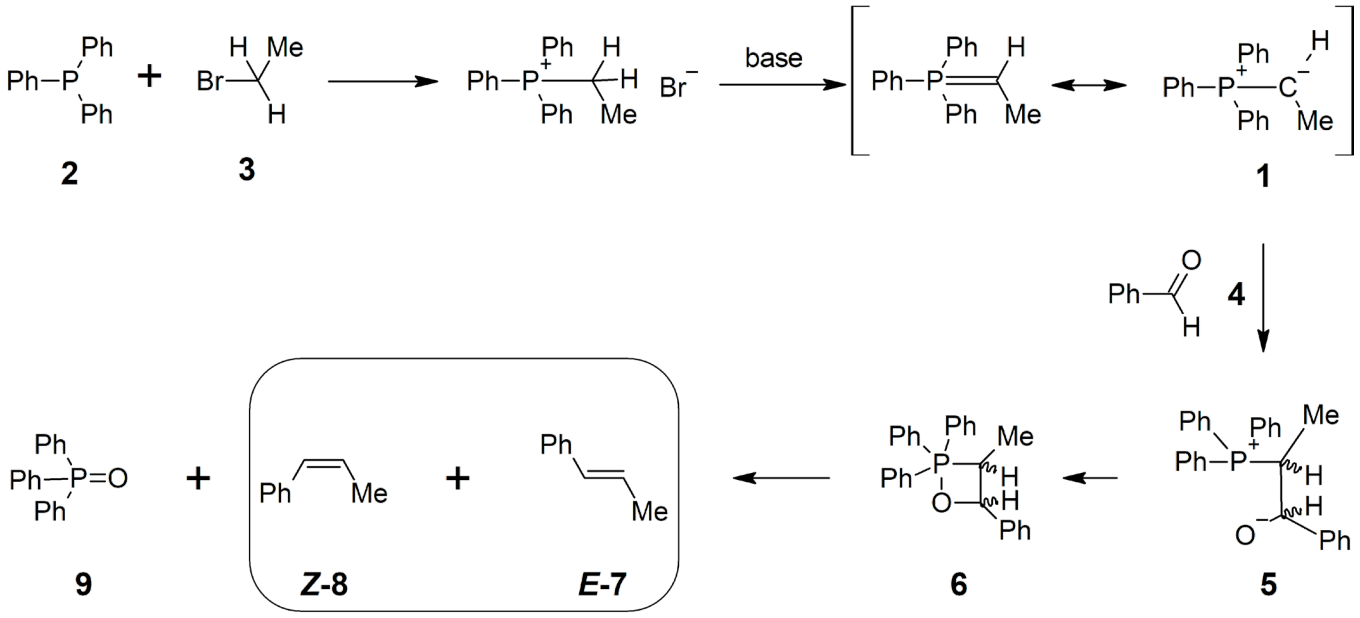
Quantifying the formation of stereoisomers by benchtop NMR spectroscopy



NMR spectroscopy is a powerful analytical tool in terms of structure elucidation and quantification of chemical moieties. In contrast with other methods, NMR does not require calibration or the synthesis of reference compounds for the analysis. These advantages become especially important when stereoisomers of a given chemical structure are present in the sample. The only alternative to NMR is chiral HPLC, however the retention times must be calibrated against the pure synthesized references which is time consuming and expensive. In this application note we demonstrate how Spinsolve™ benchtop NMR spectrometers can be used to follow on-line the kinetics of an Horner–Wadsworth–Emmons (HWE) reaction, where the stereochemical outcome needs to be monitored. The experiments performed in this work show the power of the new Spinsolve™ 80 MHz ULTRA benchtop NMR device, and its world-leading resolution, sensitivity, and stability, which enable it to identify and quantify both possible stereoisomers on-line [1].

For this work we have chosen a modified Wittig reaction. This is one of the most well-known reactions in organic synthesis and since its introduction in 1953, it has been widely used to create alkenes starting from simple carbonyl compounds, like aldehydes or ketones [2]. During this process, a carbon-oxygen double bond is converted into a carbon-carbon double bond and the oxygen is eliminated within a newly created phosphorus-oxygen double bond. Therefore, besides the carbonyl compound the phosphorus species plays a major role for the successful outcome of this type of reaction. It not only activates the donor carbon species for a nucleophilic attack, but the formation of the phosphorus-oxygen double bond constitutes the driving force of the reaction. These species are employed as phosphonium ylides **1** (often also called Wittig reagents) and can be acquired commercially or easily made in one previous synthetic step starting, for example with triphenylphosphine **2** and an alkyl bromide **3** (see Scheme 1) [3, 4].



Scheme 1: Wittig reaction scheme towards the formation of the phosphonium ylide **1** and the alkenes **7** and **8** as final products.

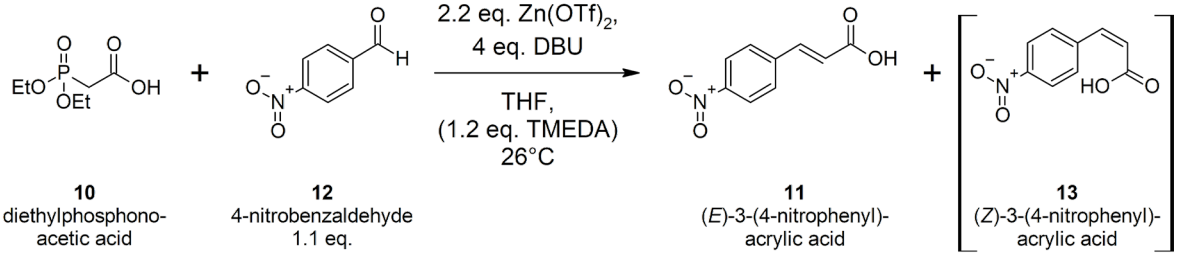
The Wittig reaction then takes place by adding a carbonyl compound like aldehyde **4** to the ylide **1**. After the nucleophilic attack of the carbanion of the ylide **1** at the carbonyl carbon of the aldehyde **4**, the betaine **5** is formed which rapidly converts to a four membered ring called oxaphosphetane **6**. In the final step, both possible alkene products **7** and **8** as well as the triphenylphosphine oxide (**9**) as a side product are formed. Due to the very strong phosphorus-oxygen double bond (575 kJ/mol), the formation of the triphenylphosphine oxide (**9**) constitutes the driving force of the reaction. Depending on which side the substituents of the products end up, one either gets the (*E*)-product **7** (substituents on opposite sides; thermodynamically favoured compound) or the (*Z*)-product **8** (substituents on the same side; kinetically favoured compound). A more detailed discussion regarding the reaction mechanism is beyond the scope of this document and the interested reader is welcome to further study the literature [5].

The outcome of the reaction in favour of one of those stereoisomers (*E*)-**7** or (*Z*)-**8** (diastereoisomers) is determined by the stereoselectivity of the process. The stereochemical outcome of the Wittig reaction highly depends on the bulk of the substituents and if the ylide **1** is stabilized or not. Over the years many optimizations towards stereoselective Wittig reactions were made and one outstanding variation is the Horner–Wadsworth–Emmons reaction (HWE reaction). In contrast to the Wittig reaction, stabilized ylides like phosphonates **10** are utilized and allow for a more selective formation of the (*E*)-product **11** (see Scheme 2) [6].

On-line reaction monitoring has proven to be a valuable tool in process control, which allows for a deep insight into mechanisms and kinetics of chemical reactions at laboratory-, as well as pilot-scale. In a recent Application Note, we demonstrated the benefits of using the Spinsolve™ Reaction Monitoring Kit following the course of an S_NAr reaction on-line employing the Spinsolve™ 60 MHz HF ULTRA system acquiring both ^{1}H- and ^{19}F-NMR spectra sequentially in a continuous mode [7].

General set-up and conditions

The reaction for this kinetic study was picked from the protocol developed by Schauer et al. [8]. They established a mild zinc promoted HWE reaction of, for example, the diprotic diethyl-phosphonoacetic acid (**10**) and 4-nitrobenzaldehyde (**12**) to yield the desired product (*E*)-3-(4-nitrophenyl) acrylic acid (**11**). Besides the formation of the (*E*)-product **11**, the corresponding stereoisomer (*Z*)-**13** can be formed additionally in such Wittig-type reactions as explained above. The authors found that the addition of tetramethylethylenediamine (TMEDA) and the utilization of bulky substituents, as with the employed aldehyde **12,** triggered an exclusive formation of the desired (*E*)-product **11**. As a solvent, tetrahydrofuran (THF) proved to be the most suited. Moreover, diazabicycloundecene (DBU) was chosen as a base and the reaction was carried out at ambient temperature (26°C). Within this study, our plan was to demonstrate one approach applying TMEDA, and a second one without, to compare the stereochemical outcome (Scheme 2).



Scheme 2: HWE reaction for the synthesis of (*E*)-3-(4-nitrophenyl)acrylic acid (**11**).

This example was studied as a batch reaction conducted in a round-bottom flask with a total volume of 100 mL. The flow-system was set up using the Spinsolve™ Reaction Monitoring Kit 2 including a glass-flow cell and a peristaltic pump connected in a closed loop with the reaction vessel. The modus operandi was chosen to be the stop-flow mode in which the reaction mixture is circulated in fast pumping intervals through the instrument with a flow rate of about 6 mL/min. The pump stops for a given time while each NMR measurement is made, and then the cycle is repeated (Figure 1).

Figure 1: Set-up of the HWE reaction in a fume hood.

Experimental procedure

Diethylphosphonoacetic acid (**10**) (0.50 g, 2.60 mmol, 1.0 eq.) was added to 12 mL of protonated THF at room temperature followed by the zinc triflate (2.10 g, 6.00 mmol, 2.2 eq.). DBU (1.55 mL, 31.5 mmol, 12 eq.) and TMEDA (0.45 mL, 3.12 mmol, 1.2 eq.) were added afterwards and the mixture was stirred at room temperature for 1 min. The reaction mixture was pumped through the Spinsolve™ system. After some test measurements, the optimal on-line monitoring parameters for this type of reaction and its concentration were determined. For instance, if solvent suppression is desired, like in this demonstration, the positions of the solvent peaks need to be determined for the reaction. The experiment loop was prepared within our RMX software tool (see all selected parameters in Figure 2). This embedded software tool enables you to choose the mode of action, to completely control the pump via the software, to acquire and record NMR spectra, and to process and evaluate the obtained data set even on-the-fly if needed (for more information regarding the RMX software tool the reader is welcome to look at this Magritek blog post) [9]. Finally, 4-nitrobenzaldehyde (**12**) (0.44 g, 2.90 mmol, 1.1 eq.) was dissolved in 3.0 mL of THF and added via a syringe to the reaction mixture. The defined experiment loop was started, and the reaction mixture was stirred overnight at room temperature. A shim protocol was performed every five measurements.

Results of Reaction Monitoring study

Before the start of the kinetic study, we measured NMR spectra of the starting material and of the reaction mixture separately at the given concentrations in protonated THF to identify the regions of interest in the spectra that could be integrated to quantify the different components of the reaction. Figure 2 shows a stacked plot of the ^{1}H-NMR data of the starting material and the reaction mixture. The spectra were recorded applying solvent suppression and carbon decoupling. The NMR spectrum of 4-nitrobenzaldehyde (**12**) is shown at the bottom of the stacked plot. The typical NMR signals in the aromatic region are visible as well as the aldehyde proton at about 10 ppm together with the residual signals of the suppressed THF resonances at 1.7 ppm and 3.5 ppm. Due to the built-in external lock system, the use of non-deuterated solvents is possible. If solvent suppression was not applied the THF signals would appear with higher amplitudes by a factor of 25. This could cause major issues to identify or quantify signals of interest near the solvent signals. The Spinsolve™ software allows up to three frequencies to be saturated simultaneously. It is also 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 higher, than the peaks of the products of interest. Here, all ^{1}H NMR spectra were acquired in the presence of carbon decoupling to remove all satellite signals.

The diethylphosphonoacetic acid (**10**) shows three peaks in the aliphatic region as well as one broad peak at about 8.1 ppm (middle spectrum of the plot). The first signal appears as a triplet at about 1.1 ppm, which belongs to the ethyl moieties (CH_3 groups). The two peaks at 2.8 ppm constitute a doublet which is caused by the coupling of the CH_2 protons in the α-position of the carboxylic acid with the ^{31}P nucleus (^{2}J = 22 Hz). The last of the three signals in the aliphatic region appears at about 4 ppm and belongs to the CH_2 groups of the ethyl moieties. In the spectrum on top, the ^{1}H-NMR spectrum of the reaction mixture can be observed (last of 500 shown). The peaks of the solvent are perfectly aligned with the peaks from the separately recorded NMR spectra of the starting material. We identified two product **11** signals between 6 and 7.8 ppm (two doublets of the vicinal α and β protons; ^{3}J = 16 Hz) that appeared, which were not overlapping either with the solvent peaks nor the signals from the aromatic region (marked in red). The aldehyde signal at about 10 ppm is still visible due to an excess of the starting material **12** (singlet; also marked in red). As stated in the literature, employing TMEDA exclusively gave access to the (*E*)-stereoisomer **11** as the sole reaction product with an *E*:*Z* ratio >95:5. As a proof of this result, NMR spectroscopy holds some key advantages compared to other analytical techniques like mass spectrometry or HPLC.

It is intrinsically quantitative and there is no need for an additional calibration of the given area of the peaks in relation to their concentrations. Moreover, due to the specific splitting patterns of the NMR peaks, it is possible to assign and identify stereoisomers with equal constitutions but different configurations. In this case with the vicinal α and β protons in *trans* position to each other, it is well known from literature that such trans-couplings of unsaturated alkenes give higher coupling constants in the range of 11–18 Hz compared to cis-couplings with only 6–14 Hz [10]. As both possible reaction products **11** and **13** are diastereoisomers and not enantiomers, their signals can be distinguished via NMR spectroscopy. The three regions that are marked in red were chosen to be followed over the course of the kinetic study of the HWE reaction (Figure 2).

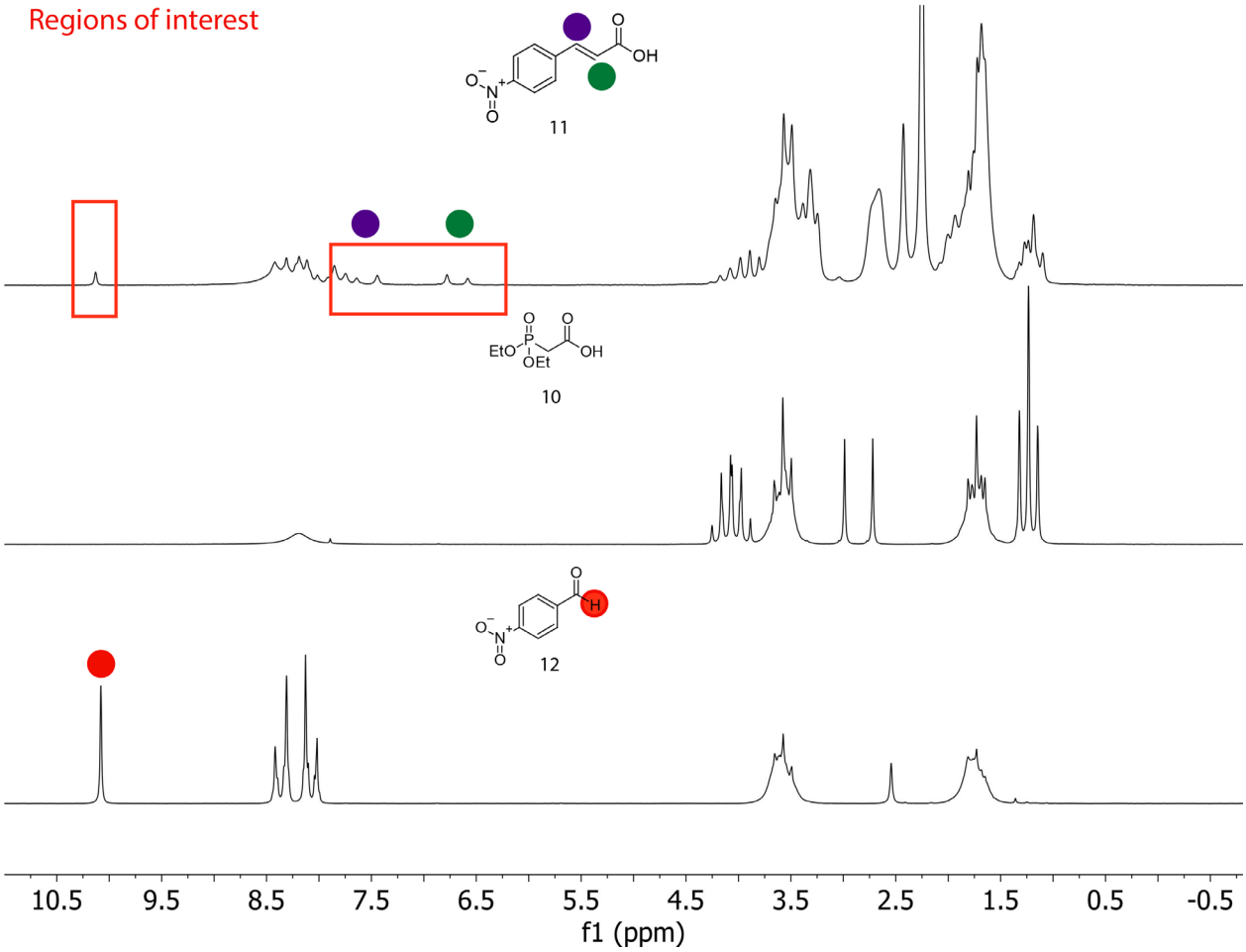


Figure 2: ^{1}H-NMR spectra (stacked) of the starting materials recorded separately as well as the reaction mixture (last spectrum of 500 shown) including the experiment parameters (8 scans, 3.2 s acq., 10 s rep., 1.7 min, 2 dummy scans, dec. on, solv. suppr. on.)

The reaction was monitored twice (once without TMEDA and once including it) by acquiring a total of 500 spectra for each trial over a time between 8.3 h and nearly 14 h. The final spectra of both trials are shown in Figure 3. The peaks of interest within the 1H-NMR spectra appear as expected in the regions between 5.5 and 8.0 ppm as well as far downfield at about 10 ppm.

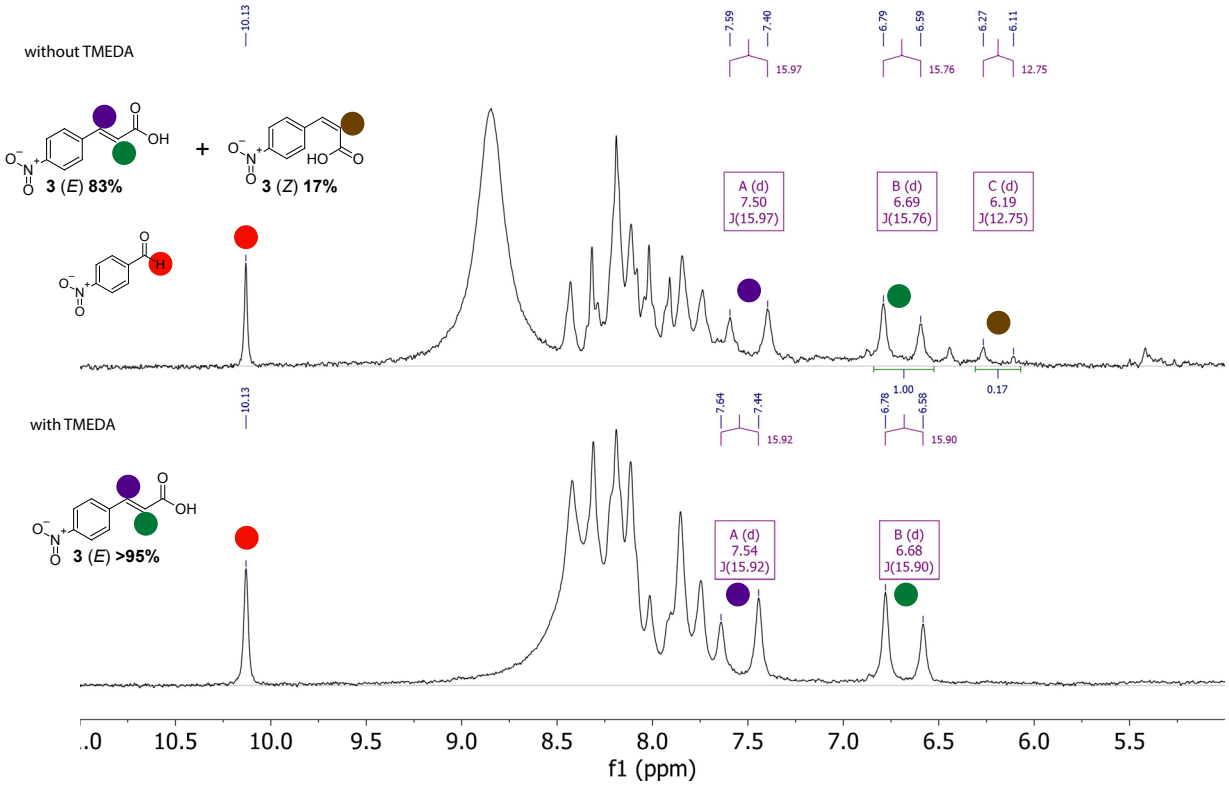


Figure 3: Last aquired ^{1}H-NMR spectra (stacked) of reaction without and with TMEDA recorded with the folloing aquisition parameters. upper spectrum: 4 scans, 3.2 s acq., 10 s rep., 90° pa, 60 s total, 13C dec. on, solv. suppr. on; bottom spectrum: 8 scans, 3.2 s acq., 10 s rep., 90° pa, 100 s total, 13C dec. on, solv. suppr. on.

As stated in Figure 2, the peaks of interest for the TMEDA trial (bottom spectrum) appeared at about 6.7 ppm and 7.5 ppm as the two doublets of the vicinal α and β protons with a coupling constant of 16 Hz. Since the aldehyde **12** was employed in excess, its singlet was still visible at about 10 ppm. The stereochemical outcome of this reaction approach was in clear accordance with the literature [8]. Only the *trans*-product (*E*)-**11** was obtained with an excess of >95%. The total measurement time of this on-line recording was 13.9 h. As already shown in Figure 2, the spectra were recorded with an applied solvent suppression of the THF peaks as well as with carbon decoupling.
In contrast to this approach, the second trial was performed without the use of TMEDA and the outcome is shown as the top spectrum in Figure 3. Here, besides the two doublets of the vicinal α and β protons from product **11** with a coupling constant of 16 Hz, we also obtained additional doublets with significantly smaller integrals at about 6.2 and 6.6 ppm. The coupling constants were determined to be 13 Hz and the integral value was 0.17 for the doublet at 6.2 ppm (reference integral of 1 for the doublet at 6.7 ppm). In agreement with literature, this set of doublets belongs to the cis-product (*Z*)-**13**, which is the diastereoisomer of the major product **11**. This side product (*Z*)-**13** was obtained in a ratio of 83 to 17% in favour of the trans-product (*E*)-**11**. The coupling constant of 13 Hz is proof of that outcome and a clear sign for the *cis*-protons in the α and β positions of the kinetic product (*Z*)-**13** [8, 10]. The authors of the scientific paper we employed for this study state that TMEDA is believed to play a crucial role in the stereochemical outcome of this HWE reaction. It can act as a chelating agent and boost the stereoselectivity towards the thermodynamic product **11** [8]. Except for the number of scans, all parameters were left the same as for the first approach employing TMEDA.

We could demonstrate the successful second approach of this reaction with an even lower number of scans of 4. The signal to noise ratio was still more than sufficient for the given concentrations employing the Spinsolve™ NMR system. An additional broad singlet appeared at about 8.8 ppm, which we believe corresponds to the acidic proton of the carboxylic moiety of the diethylphosphonoacetic acid (**10**).
When applying TMEDA, which can also act as a base, no broad singlet is visible. The aldehyde **12** proton is again visible due to its addition in excess. Since the products **11** and **13** only consist of protons from the aromatic region and protons of the unsaturated double bond, no focus was set on the aliphatic region. Nevertheless, the solvent suppression of the THF signals would allow for a clean assignment of the diethyl-phosphonoacetic acid (**10**) and its disappearance (see mid spectrum in Figure 2).
Figure 4 shows the complete stacked plot of the trial without adding TMEDA including the trends of the obtained ^{1}H spectra over time for the regions of interest.

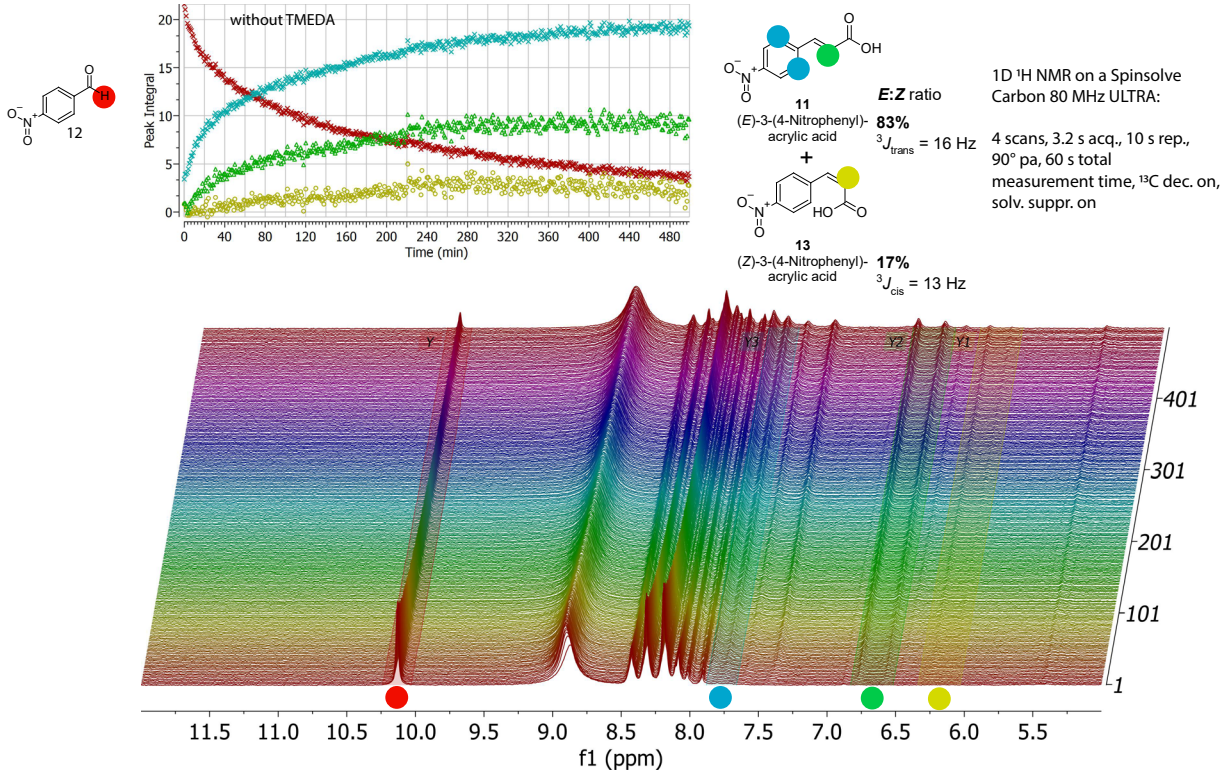


Figure 4: ^{1}H-NMR spectra (stacked plot at the bottom; due to visibility reasons a decimation of 2 was applied) of the kinetic study with an overall spectra number of 500 including the trends of the integrals over time of the regions of interest.

The stacked plot of 500 spectra recorded over a total measurement time of 8.3 h is shown at the bottom of Figure 4. Due to visibility reasons only every second spectrum is shown in this stack. The regions of interest are marked in colour and belong to the two expected stereoisomers (*E*)-**11** (green and blue) and (*Z*)-**13** (brown). The aldehyde **12** signal was also followed over time and is marked in red here. Additional to the signals identified in Figure 3, two proton signals from the aromatic region were assigned as belonging to the *trans*-product (*E*)-**11** and are marked in blue. At the top of Figure 4, the trends of the regions of interest are displayed over time. For the *trans*-product (*E*)-**11**, two regions of interest are marked in green and blue.

Considering that the peak at about 7.8 ppm (blue) should have twice the peak integral as the peak at 6.7 ppm (green), since it belongs to two protons from the aromatic ring compared to one proton from the α position, the results obtained match this expectation very well with peak integrals increasing towards 20 (blue line increasing) and 10 (green line increasing). Regarding the stereochemical outcome of this HWE reaction, the regions marked in green and brown were considered. These regions belong to both the α protons (as described in Figure 3) of the reaction products **11** and **13** and their peak integrals differ roughly by a factor of 5, which is in agreement of the determined ratio in Figure 3. The trend lines are increasing towards 10 (green trend line) and 2 (brown trend line) respectively. For the identification and assignment of the diastereoisomers, the coupling constants were considered. The red trend line displays the decrease of the aldehyde **12** proton at about 10 ppm. Since this moiety was used in excess, the signal is still visible at the end of recording. Moreover, the main goal of this study was not to drive this reaction towards completion but to study the stereochemical outcome of this reaction in detail and assign its ratio.
The ^{1}H NMR data in the stacked plot is of very high quality as visible in Figure 4. Nearly all peaks are aligned perfectly over the course of 500 measurements. Only the broad singlet peak at 8.8 ppm is shifting slightly within the first five measurements. This peak belongs to the acidic proton of the carboxylic moiety of the diethylphosphonoacetic acid (**10**) and is believed to move due to its dynamic property and pH changes. No manual alignment was performed and only the standard processing options were applied like apodization and baseline correction. These results demonstrate for the first time a Spinsolve™ benchtop NMR system being employed to not only follow the course of the HWE reaction during on-line monitoring experiments but also to study the stereochemical outcome of this type of reaction. We were able to identify both diastereoisomers that were made during this reaction and we could quantify their ratios. The results were in complete agreement with the literature sources stated.

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