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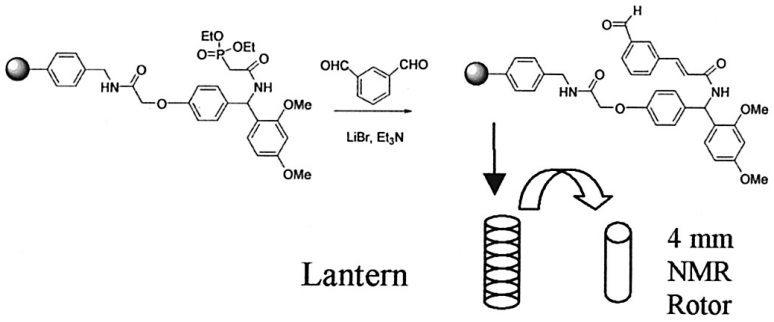

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Monitoring of Solid-Phase Organic Synthesis on Macroscopic Supports by High-Resolution Magic Angle Spinning NMR

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In this paper we demonstrate the efficiency of high-resolution magic angle spinning NMR to monitor solid-phase organic chemistry on macroscopic systems such as Synphase lanterns. The use of the LED sequence eliminates the peaks due to the use of protonated solvents and was also sufficient to decrease the signals due to the matrix. As a direct result, we established that reaction kinetics on the lantern proved to be significantly more rapid than on an equivalent polystyrene resin. More generally, the macroscopic nature of the support facilitates both sample preparation and spectral recording and hence opens up the perspective of an automated on-line analysis in combinatorial chemistry.

Introduction

Aimed mainly at the acceleration of drug discovery programs, a variety of techniques for the rapid simultaneous synthesis of a large number of compounds are now being used worldwide.[1] Whereas solution-phase organic synthesis still is the method of choice for a number of reasons varying from the wide range of accessible reactions to the existence of reliable in-process control methods,[2] the urge for increased complexity through multistep reactions recently has brought solid-phase organic synthesis to the forefront in the combinatorial chemistry effort. Solid-phase organic chemistry (SPOC) is traditionally performed on cross-linked polymeric beads typically 100 μm in size and with a charge of 1−10 nmol per bead, but macroscopic supports have recently started their entrance in the field.[3] Especially in the automation procedure, the straightforward handling of these supports gives them a distinct advantage over their beaded counterparts. The possibility of modeling them in different shapes has very recently been used as a very simple tool in the deconvolution process,[4] and it was demonstrated that resin sintering within an inert polymer matrix is a general method that can be applied to basically all common resin types.[5]

In situ analysis of the grafted molecules on such supports has been performed by mass spectroscopy,[6] FTIR,[7] and, recently, by NMR. Using a full crown (6 mm in diameter, 20 mm in height, and a load of 18 μmol) in a 7 mm cross polarization magic angle spinning (CPMAS) probe, Shapiro et al. recorded workable one- and two-dimensional spectra after extensive exchange of the protonated reaction solvent to the equivalent deuterated one.[8] More recently, Gerritz et al. shaved thin pieces off the crown surface and introduced them after rinsing and washing with deuterated solvent into a 4 mm nanoprobe.[9] Whereas the nanoprobe (or equivalent 4 mm high-resolution magic angle spinning (HRMAS) NMR probe) is more conventional than the previously used 7 mm probe, the shaving procedure was inherently destructive and did not allow for further chemical transformations.

We show here that satisfactory spectra can be obtained on SynPhase lanterns[10] in protonated solvent by using diffusion-filtered HRMAS NMR as developed in our laboratory.[11] Compared to our previous work on reaction monitoring on a resin sample,[12] several distinct advantages of the macroscopic support can be pointed out: (i) washing leads to reaction quenching for the whole sample, avoiding thereby the time constraints for spectral recording; (ii) the full sample is in the rotor, so one does not face statistical sampling problems; (iii) contrary to the case of resin samples, where the handling of milligram quantities always leads to losses, working with a macroscopic lantern support does not suffer from any sample loss. In the present paper, we will address three different aspects of analysis: the identification of a reaction product by one- and two-dimensional NMR, the reaction monitoring on a minute time scale, and the possibilities of impurity detection.

Results

We followed a Wittig−Horner condensation in order to compare our results with those of the same reaction on a standard resin.[12] The terephthaldehyde was reacted with the phosphonodiester, and we monitored the appearance of a tethered aldehyde signal around 10 ppm (Scheme 1). Because the diameter of the lantern was too big to fit in a 4 mm rotor, we had cut the lantern into four pieces. Whereas this clearly is not satisfactory, it did allow us to maintain the macroscopic nature of the support in the sense that we could easily remove it from the rotor and reintroduce it in the reaction vessel. Still, suitably sized macroscopic supports should be soon developed by the manufacturers, and the

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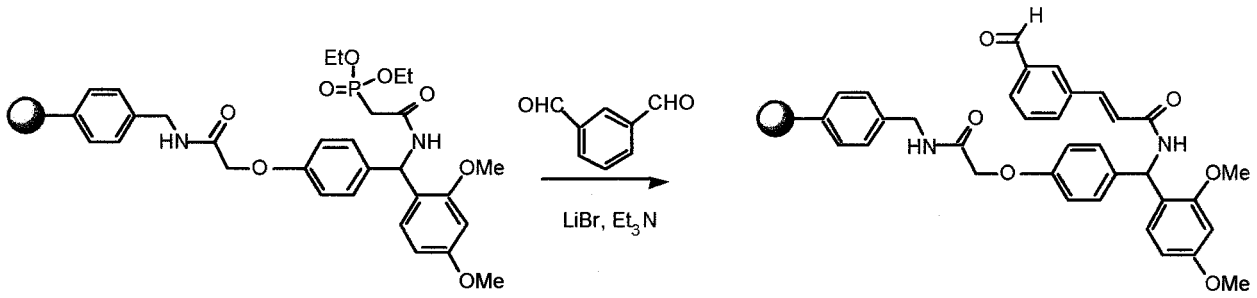
Scheme 1



sintering procedure that was very recently described[5] is definitely able to make macroscopic supports in the shape and size of a rotor.

We first recorded spectra on the sample in DMF-d_7 in order to find optimal NMR conditions for the subsequent reaction monitoring. Regular single-pulse spectroscopy gave good results, but in order to optimize the NMR parameters for subsequent reaction monitoring, we equally optimized NMR parameters for the diffusion-filtered experiment as implemented by the LED sequence.[14] In agreement with previous results on the resin samples,[12] a 28 ms diffusion delay and gradient strengths of 35 G/cm over 5 ms gave satisfactory results. The broad signals of the matrix, previously suppressed by the use of a CPMG pulse train[15] or by the combined use of CPMG and diffusion filtering,[16] were here largely eliminated by the T_2 relaxation during the gradient delays. After this optimization step on the sample in DMF-d_7, we recorded a spectrum of the lantern in protonated solvent, and the diffusion filter proved its efficacy to suppress the signals from the solvent and to attenuate the broad lines of the matrix (Figure 1, bottom). Not amazingly,

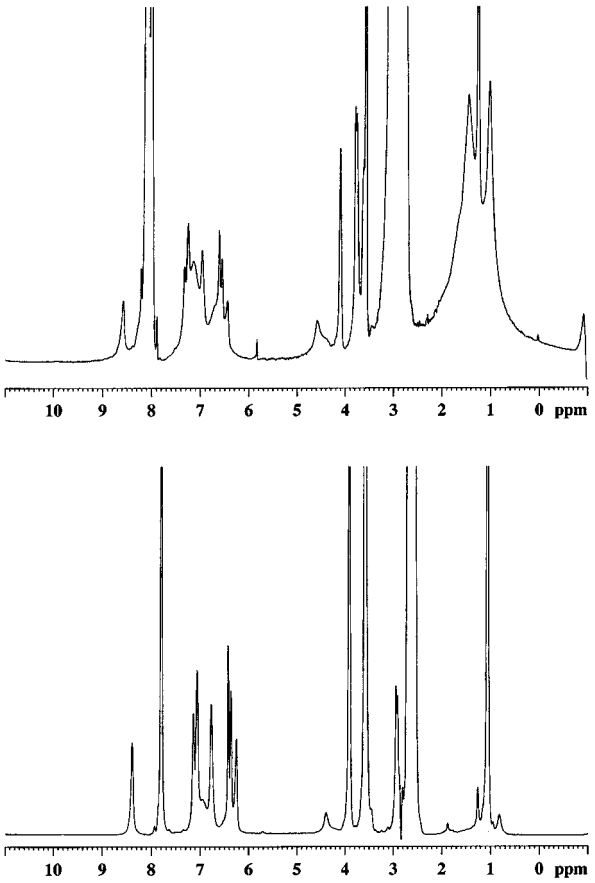


Figure 1. 1D spectra of a quarter of a commercialy available SP-PS-D-RAM lantern swollen in DMF-h7, before any reaction. The spectrum was recorded with 16 scans on a Bruker DMX 600 MHz spectrometer equipped with a 4 mm single axis gradient HRMAS NMR probe. The single pulse experiment (top) has intense signals of the protonated DMF and broad lines from the support at 1.5 and 7 ppm. Both spectral artifacts are largely reduced in the diffusion-filtered experiment (bottom).

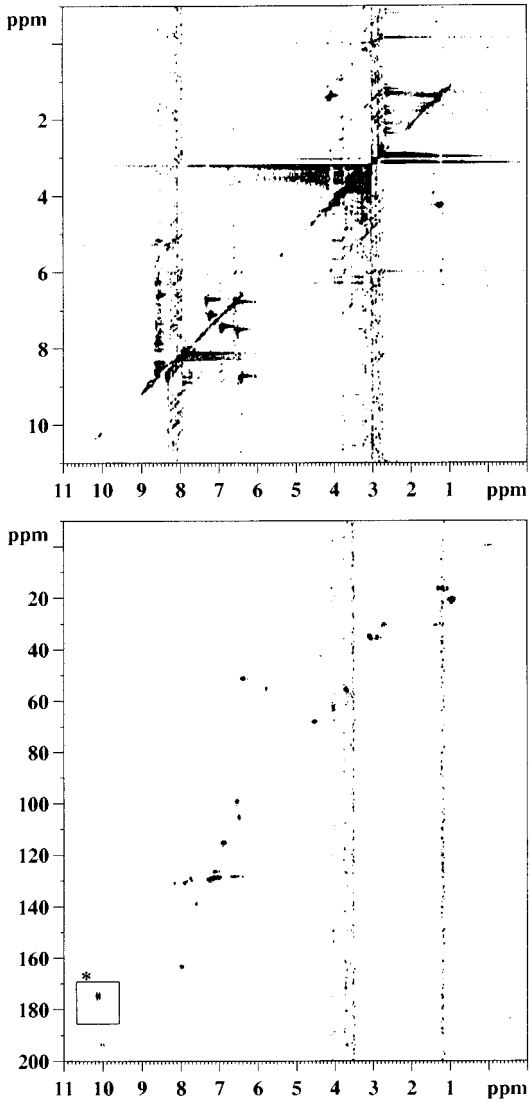


Figure 2. 2D spectra on the final sample after the Wittig−Horner condensation. The TOCSY spectrum (top) was recorded in protonated DMF and used diffusion filtering. The $^1H-^{13}C$ HSQC spectrum (bottom) was recorded on the same sample swollen in DMF-d_7. The aldehyde correlation peak visible at 10 and 192 ppm is magnified in the box (∗).

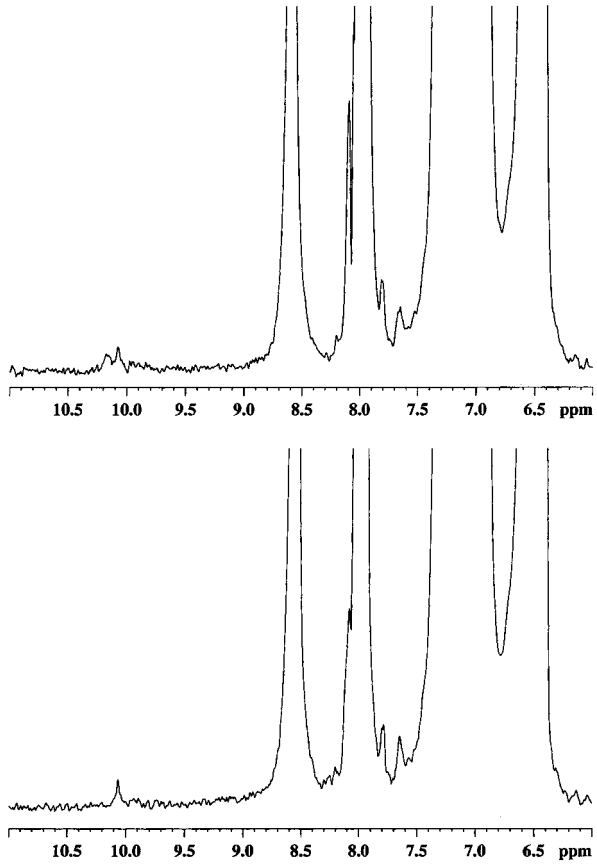


Figure 3. Diffusion-filtered 1D spectra after a single washing (top) and triple washing by flushing a large volume of DMF over the lantern in a syringe (bottom). The slowly diffusing, nonreacted aldehyde in the polystyrene matrix gives an additional signal at 10.2 ppm in the top spectrum, which disappears completely after thorough washing.

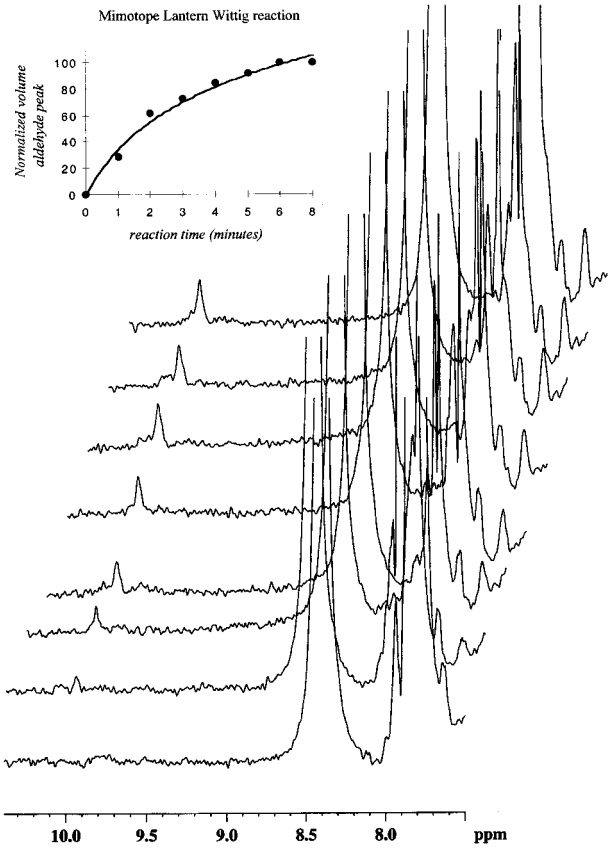


Figure 4. Diffusion-filtered 1D spectra recorded for every minute of the reaction (see text). The resulting buildup curve that shows the reaction progress is shown in the inset.

the signals of the tethered linker in the single-pulse spectrum were of higher intensity than the equivalent signals in a resin sample. If we estimate that our sample was exactly one-quarter of the full lantern, we have 8 μmol of the linker moiety in the rotor, which would correspond to 20 mg of resin beads with a standard charge of 0.4 mmol/g. We further recorded homonuclear total correlation spectroscopy (TOCSY) and heteronuclear single quantum coherence (HSQC) spectra on the final sample after the Wittig−Horner condensation (see below) in order to assign all the resonances on the lanterns, demonstrating that complete identification of the reaction products can be readily achieved (Figure 2).

The Wittig−Horner condensation was performed in a regular Eppendorf tube, using directly 10 equiv of base and catalyst.[12] We introduced the lantern quarter in a solution of aldehyde and then added the solution of catalyst and base. After removing the lantern from the reactor and washing it several times with DMF in a syringe in order to quench the reaction, we started the spectral recording. The washing step proved to be very important for removing all the unreacted compounds. After a simple step of washing, soluble aldehyde that has not reacted but is slowly diffusing into the polymer matrix can still be seen next to the tethered one in the top spectrum. After triple rinsing by flushing the lantern by a large volume of DMF in a syringe, the additional signal disappears completely (Figure 3). Because the lantern represents the whole reaction vessel, washing efficiently quenches the complete reaction, reducing all time constraints for spectral recording. The absence of time constraints allowed the use of 256 scans in order to obtain a good signal/noise ratio on our sample, thereby improving the subsequent quantification by spectral integration.

After each spectra, we put the lantern back in the reaction vessel and let the reaction continue for another minute. Reaction progress was monitored by integrating the aldehyde peak without any need for integral referencing because the total sample was monitored in the NMR spectrum (Figure 4). However, the resulting reaction curve does not yield the absolute amount of material that has formed over time, and similar to our previous results, calibration of the total quantity of material on the lantern requires the use of an internal proton as reference.[12] The shape of the curve is in a good agreement with our previous results, but the kinetics proved to be much faster. This result can be linked to structure of the lantern itself, which in the absence of cross-linking of the uniform 50 μM polystyrene graft allows a better diffusion of the reactive species inside the matrix.

Impurity detection and identification by HRMAS NMR was previously shown to be a very sensitive and precise method (on a sample composed of a regular resin).[16] Here, we investigated the same problem on the macroscopic lantern support by coupling simultaneously two different aldehydes in a well-defined ratio. To minimize differential reactivities between the two compounds, we first coupled one equivalent composed of 20% terephthaladehyde and 80% 3-cyclohexen-

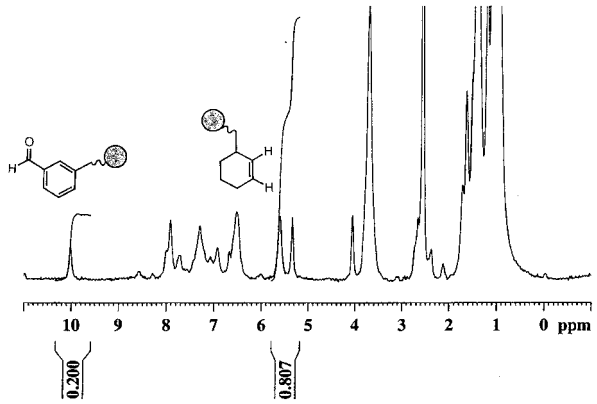


Figure 5. Single-pulse spectrum with a 3 s relaxation delay of a phosphonodiester-containing lantern that reacted with 20% terephthaladehyde and 80% 3-cyclohexen-1-aldehyde. Integrals quantify the olefin proton signal between 5 and 6 ppm, representing the major species, and the characteristic aldehyde signal at 10 ppm for the minor one.

1-aldehyde on the same lantern. After a 10 min delay corresponding to the previously established time scale of the reaction, we added one more equivalent of the same mixture. The 1D spectrum of the resulting sample allowed identification of both species through the olefin proton signal between 5 and 6 ppm for the major species and the characteristic aldehyde signal at 10 ppm for the minor one (Figure 5). The integration of the two different peaks yielded values of 81% for the 3-cyclohexen-1-aldehyde and 19% for the terephthaladehyde, in good agreement with the theoretical percentage introduced. This establishes convincingly that impurity detection by HRMAS NMR is equally valid for the macroscopic solid supports.

Conclusion

Our study has shown that product detection, identification, and quantification on a lantern system by HRMAS NMR all are feasible. The use the LED sequence allows us to work in protonated solvent and suppresses simultaneously the signals due to the matrix. As a direct result, we established that the reaction kinetics on the lantern proved to be significantly more rapid than on an equivalent polystyrene resin. Impurity detection and identification were equally satisfactory. More generally, the macroscopic nature of the support facilitates both sample preparation and spectral recording and hence opens up the perspective of an automated on-line analysis in combinatorial chemistry. The development of slightly smaller lanterns will significantly advance the perspectives of automation of reaction monitoring in combinatorial chemistry.

Experimental Procedures

NMR Experiments. After loading the piece of the lantern (after washing three times with protonated DMF) in the 4 mm rotor, DMF-d_7 was added to solvate the lantern. Tetramethylsilane (TMS) was added as an internal reference to the solvent before the lantern swelling. All NMR experiments were performed at 298 K on a Bruker DMX 600 MHz spectrometer (Bruker Spectroscopin, Germany) equipped with a 4 mm HRMAS probe using a 6 kHz spinning rate. The spectra from the single-pulse experiments were recorded with 64 scans, and the spectra from the diffusion-filtered experiments (LED) were recorded with 256 scans. Gradient parameters for the LED-based sequence were as previously described.[10] Spectral processing and integration were performed with XWINNMR from Bruker.

Wittig Condensation. After the Fmoc deprotection of the lantern with a standard protocole, the condensation of diethylphosphonoacetic was performed. With respect to the lantern charge a total of 5 equiv of acid was dissolved in DMF and activated by adding 5 equiv of HBTU, 5equiv of HOBt, and 10 equiv of DIEA. After a short delay of activating time, the solution was poured onto the lantern and shaken for 2 h. The coupling of the acid was performed a second time to ensure a complete reaction.

Horner−Emmons Reaction Monitoring. The lantern was loaded in a reaction vessel, and 5 equiv of therephthaldehyde in DMF was added. The reaction was started by the addition of the appropriate amount of LiBr/Et_3N. Before each spectrum was recorded, the piece was removed from the reaction medium, washed three times with protonated DMF, and then loaded in the 4 mm rotor. After each spectrum, the lantern was put back in the reaction vessel to let the reaction proceed. To check the formation of any byproducts such as dimeric species, we recorded a mass spectrum after TFA cleavage from the lantern. The mass spectrum only showed the peak corresponding to the expected product, whereas no peak corresponding to the dimer was detected.

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