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J. Comb. Chem., **2000**, 2 (3), 293-296 • DOI: 10.1021/cc0000038 • Publication Date (Web): 12 April 2000

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SPEEDY: Spin–Echo Enhanced Diffusion Filtered Spectroscopy. A New Tool for High Resolution MAS NMR

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Received January 10, 2000

The pulsed field gradient diffusion edited experiment, bipolar LED, has been combined with the Carr–Purcell–Meiboom–Gill (CPMG) spin–echo sequence for the analysis of solid phase resin samples. Spin–echo enhanced diffusion filtered spectroscopy (SPEEDY), when optimized, filters both the compounds that demonstrate fast diffusion rates as well as the compounds that demonstrate fast T_2 relaxation rates. Using this technique, compounds that are not covalently attached to the resin are not observed and contributions from the resin matrix are greatly attenuated. The interpretation of the resulting spectrum is more readily accessible. This technique lessens the importance of completely removing reaction residues or the wash solvents simply for analytical evaluation. The utility of the combined filtering scheme was demonstrated by the implementation into a NOESY sequence.

Introduction

The generation of compound libraries using solid phase synthesis has had a significant impact on combinatorial chemistry. The advantages of using solid phase synthesis have been well established in terms of compound manipulation and product purification. While purification of the compounds has been simplified by the use of solid supports, the use of ^1H NMR for monitoring stepwise synthesis without cleavage from the resin still has interpretation problems.

High resolution magic angle spinning NMR (hr-MAS) has been established as a suitable method for the analysis of resin bound molecules.^{1,2} The major drawback with the analysis of resin, using hr-MAS, has been the difficulty in the interpretation of the spectra obtained. The complexity of the spectra obtained can be divided into two parts: contribution of signals that arise from the resin matrix including linkers and signals that result from noncovalently attached compounds that were not removed during the washing stages of the resin.

Methodology has been developed which addresses both of these problems on an individual basis. The contributions from the resin matrix can be attenuated by the use of a spin–echo sequence,³ presaturation,^{4,5} 2D J -resolved,⁶ or SECSY⁷ experiment. Recently, diffusion based experiments have been applied in hr-MAS analysis for the attenuation of compounds not attached to the resin.^{8,9} Evaluation of resin reactions thus required the acquisition of multiple experiments for adequate product confirmation.

It was the goal of this work to combine the benefits of the diffusion edited sequence with the Carr–Purcell–Meiboom–Gill (CPMG) sequence to yield a single experiment that can be readily interpreted. The spin–echo enhanced diffusion filtered spectroscopy (SPEEDY) described here utilizes the bipolar longitudinal eddy current delay (bipolar

LED) sequence followed by the CPMG spin–echo sequence that results in a single spectrum that contains only resonances from the compounds attached to the resin and minimal contributions from the resin matrix. The benefits of the combined filtering scheme were then extended to the 2D NOESY experiment.

Experimental Section

All experiments were performed using a Bruker DMX500 (^1H : 500.13 MHz) equipped with an Accustar III gradient pulse amplifier and a high resolution (^1H , ^{13}C) 4 mm MAS probe with Z -axis gradient. Fmoc-isoleucine Wang resin (1–2 mg) was placed into a 4 mm ZrO rotor equipped with a KEL-F spherical insert. Benzoyl-DL-phenylalanine (1.5 mg) was dissolved into DMF- d_6 (1 mL), and the resulting solution (15 μL) was added to the rotor.

The rotor was placed into the hr-MAS NMR probe and the sample spun at 4000 Hz without temperature regulation. The one-dimensional experiments were acquired with 16K data points and spectral widths of 7500 Hz using the following parameters. The CPMG experiment was acquired with $\tau_{\text{cp}} = 0.5$ ms and $n = 70$. The one-dimensional bipolar LED CPMG experiment was acquired according to the pulse sequence in Figure 1a using the following parameters: $\tau_{\text{cp}} = 0.5$ ms, $n = 70$, the gradients used were rectangular shaped with duration of 2 ms and strength of 38 G/cm.

The NOESY experiment used was phase sensitive (TPPI) with homospoil pulses during the mixing time. The data matrix was 4096 data points (t_2) \times 128 data points (t_1), 16 scans per increment, and spectral widths of 7500 Hz in both dimensions. The gradients used for this experiment were sine shaped each with duration of 1 ms and strengths of 26 G/cm and -26 G/cm.

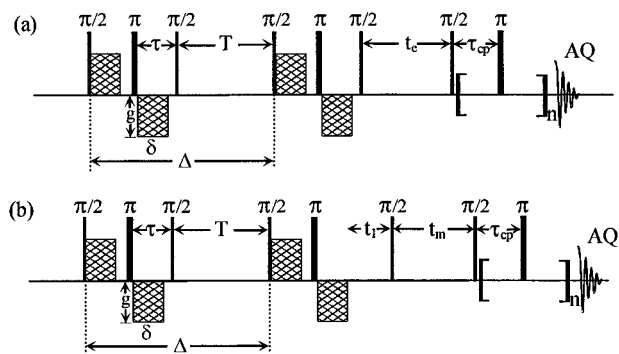


Figure 1. (a) Pulse sequence of bipolar LED-CPMG. Typical bipolar LED phase cycling is used. The 180° pulses in the CPMG pulse train are phase shifted by 90° with respect to the preceding 90° pulse. (b) Pulse sequence of bipolar STE-NOESY-CPMG. The phase cycle is the same as that for part a. Phase sensitive TPPI is applied to the third 90° and the second 180° pulses.

The STE-NOESY-CPMG experiment was acquired according to the pulse sequence in Figure 1b. The data matrix was 4096 data points (t_2) \times 128 data points (t_1), 64 scans per increment, and spectral widths of 7500 Hz in both dimensions. The gradients used for this experiment were rectangular shaped each with duration of 2 ms and strengths of 38 G/cm. The mixing time was $t_m = 650$ ms, $\tau_{cp} = 0.5$ ms, and $n = 70$.

Results

Benzoyl-phenylalanine was added to Fmoc-isoleucine Wang resin to simulate the inefficient washing of the solid phase resin. Parts A–C of Figure 2 represent the typical one-dimensional proton experiments required for an initial evaluation of a solid phase organic synthesis (SPOS) resin utilizing ^1H hr-MAS NMR. Figure 2A shows the proton spectrum obtained from the standard single pulse experiment. The broad resin resonances clearly obscure and distort important portions of the spectral window. Attenuation of the resin matrix can be accomplished by the use of the CPMG experiment. Figure 2B shows the resulting spectrum after the application of the CPMG. The CPMG echo time was selected to attenuate the broad resin signals. The methylene groups from the isoleucine can then be observed as well as the phenylalanine β - CH_2 . Interpretation of this experiment would suggest that either the isoleucine or the phenylalanine was attached to the resin. Figure 2C shows the spectrum that was obtained using the bipolar LED experiment. The bipolar LED experiment attenuates compounds with faster diffusion coefficients; therefore, compounds that are not attached to the resin can be attenuated. The solvent, DMF, and the benzoyl phenylalanine resonances have been effectively removed, demonstrating that the Fmoc-isoleucine was the compound that was covalently attached to the Wang resin. The Wang resin resonances, however, were not attenuated and continue to obscure some of the isoleucine resonances. The combined bipolar LED CPMG experiment, Figure 2D, incorporates the advantages from both experiments. The solid phase matrixes as well as the compounds not attached to the resin have been attenuated, resulting in the conclusion that Fmoc-isoleucine has been attached to the Wang resin.

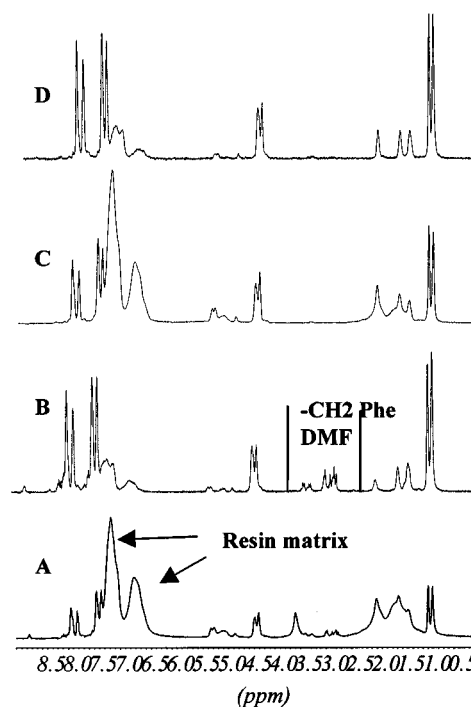


Figure 2. Fmoc-isoleucine Wang resin, benzoyl phenylalanine in DMF, 4000 Hz. (A) single pulse experiment, $n_s = 32$. (B) CPMG 70 ms spin-echo, $n_s = 32$. (C) Bipolar LED experiment with $\delta = 2$ ms, $g = 38$ G/cm, $n_s = 64$. (D) Bipolar LED-CPMG experiment with $\delta = 2$ ms, $g = 38$ G/cm, $n_s = 64$ and total spin-echo time of 70 ms.

Figure 3A shows the results obtained using a gradient modified NOESY experiment. Contributions from the resin matrix can be identified as the broad cross-peaks. The results that were obtained using the bipolar STE-NOESY-CPMG experiment are shown in Figure 3B. The observation of intramolecular NOEs of covalently attached compound (isoleucine), which are hidden by resin and nonbinding compound signals in the regular NOESY spectra, can be easily identified in the modified NOESY spectra.

Discussion

High resolution MAS NMR is a useful analytical tool for the analysis of solid phase synthetic resin. The use of ^1H hr-MAS NMR for the analysis of solid phase synthesis resin has been hampered with two major obstacles, the resonances from the solid phase matrix and contributions from compounds that are not related to the resin or the compound of interest.

Within the MAS rotor, one can distinguish three different and observable NMR domains. The three different domains are the solid phase support, the compound that is attached to the resin (including the linker), and the compounds that are free in solution (including solvent). The three domains possess different physical properties that can be exploited by NMR experiments to obtain interpretable NMR spectra. The solid support is typically a macromolecular polymer support. In terms of the NMR time scale, the T_2 relaxation rates for macromolecules are fast which yield broad lines in the proton spectrum. The one-dimensional CPMG experiment has been demonstrated to attenuate the broad signals from the solid support. The resulting spectrum (Figure 2B) has

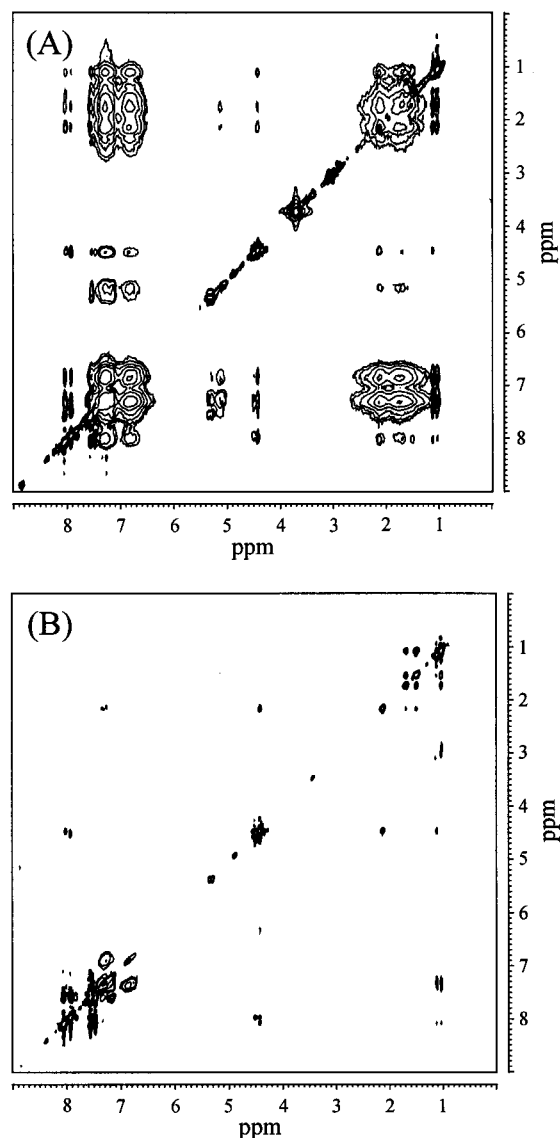


Figure 3. Fmoc-isoleucine Wang resin, benzoyl phenylalanine in DMF- d_7 . (A) NOESY spectrum $t_m = 650$ ms, $ns = 16$. (B) Bipolar STE-NOESY-CPMG spectra: $\delta = 2$ ms, $g = 38$ G/cm, $t_m = 650$ ms, $ns = 64$, $\tau_{cp} = 0.5$ ms, and $n = 70$.

been utilized to attenuate the resin resonances yielding spectra more amenable to interpretation. However, the remaining resonances can be attributed to either the covalently bound product or impurities such as reaction starting material or reaction solvents that were not removed during the washing steps.

The compounds that are free in solution are generally small molecules, reaction solvents, residual starting material, or reaction byproducts. These small molecules possess fast self-diffusion coefficients and can be attenuated by the use of a NMR diffusion filtered experiment. The compounds that are attached to the resin exhibit slower T_2 relaxation rates in comparison to the solid phase matrix and slower self-diffusion rates in comparison to the compounds that are free in solution. Diffusion filtered experiments were recently implemented that filter the spectrum for compounds that are not attached to the solid phase matrix. Residual solvents, starting material, or reaction byproducts, which may not have been washed out of the resin, will not be observed.

The combination of the diffusion filtered NMR experiment and a spin-echo experiment would then select only the resonances that exhibit medium self-diffusion rates and medium T_2 relaxation rates. The resultant spectra (Figure 2D) can then be interpreted as compounds that are attached to the resin.

Although the proton spectra provide adequate information concerning solid phase resin reactions, 2D experiments may be required to provide conformational information. The traditional NOESY experiment can be applied toward the analysis of solid phase resin reactions to yield conformational information; however, contributions from the resin matrix overwhelm the spectra (Figure 3A). The benefits in selecting for compounds that exhibit medium T_2 relaxation rates and medium diffusion coefficients can be shown by extending the concept toward 2D experiments. The modified NOESY experiment incorporates a stimulated echo diffusion filter as well as a CPMG pulse train (Figure 1b). The resultant spectrum (Figure 3B) filters for compounds that are attached to the resin, reducing the contributions from the solid support and removing the compounds that are free in solution. Clearly the aliphatic region, 2.5 ppm – 1 ppm, in the standard NOESY is dominated by interactions from the Wang resin; but the STE-CPMG-NOESY experiment attenuates the resin signals, and correlations from the isoleucine side chains can be observed.

Conclusion

The success of this experiment takes advantage of two physical properties inherent within the resin environment. Compounds that are not attached to the solid phase matrix are typically unreacted starting material, residual solvents, or reaction byproducts, all of which are small molecular weight compounds and exhibit relatively fast diffusion rates. Conversely, the resin matrix is composed of polymer like material and exhibits fast T_2 relaxation times. The development of an experiment that exploits both of these properties thus allows a fast and easy method for the analysis of compounds that are attached to the resin. The diffusion edited portion of the pulse sequence attenuates these types of molecules, thus filtering the spectra of the compounds not covalently attached to the resin. The CPMG spin-echo pulse train in a similar fashion attenuates resonances with short T_2 relaxation rates or generally the solid phase matrix.

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CC0000038