Efficient Suppression of Solvent Resonances in HR-MAS of Resin-Supported Molecules

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Combinatorial chemistry is a very promising approach to accelerate the drug discovery process,[1] and solid-phase synthesis is emerging as a major tool to assemble diverse compound libraries, especially since it provides a methodological approach to the synthesis of drug-like small organic molecules. However, organic reactions on solid supports often require conditions different from those in solution, and chemistry validation and optimization remain one of the time-limiting steps in the development of new libraries. One of the disadvantages in this process is the lack of universal analytical methods for on-resin quantitative analysis of the solid-supported compounds, both at intermediate stages and of the final products.

FTIR microspectroscopy can be used to monitor the disappearance of the starting material accompanied by the appearance of a product absorption;[2] however, no direct information on the whole molecule is obtained, and an appropriate functional group for IR-detection is required. The combination of correct swelling conditions and high resolution magic angle spinning NMR (HR-MAS) offers a new alternative analytical tool to study in atomic detail resin-bound molecules, without the need of a destructive cleavage step. First applied in the framework of organic synthesis, where complex multistep reactions could be successfully followed,[3] its application to the solid-phase synthesis of peptides has allowed correlation of the loss of microscopic mobility of the chains to coupling difficulties.[4] Whereas the quantity of material needed to obtain an acceptable proton spectrum in one minute is now reduced to less than 1 mg (Figure 1), the need to swell the resin in a deuterated solvent remains a major problem. A sample preparation requiring steps of washing with a volatile solvent, before drying under vacuum and subsequent swelling in the appropriate deuterated solvent, is time-consuming and incompatible with the in situ characterization of resin-bound structures directly from the reaction vessel. Deuterated solvents are required because most organic solvents show more than one proton resonance (and their corresponding ^{13}C satellites) that can only be suppressed by sophisticated solvent suppression methods combining selective pulses and pulsed field gradients.[5] We demonstrate here how to suppress effectively **all** solvent resonances, based on the differential diffusion behavior of the solvent and the attached molecules.

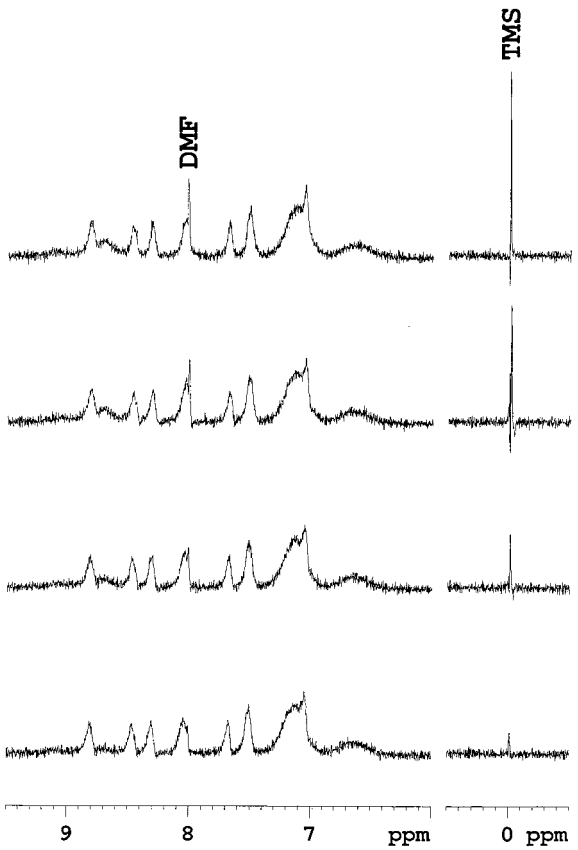

Figure 1. Proton spectra at 300 MHz of 0.15 mg of Ile-Leu-Asn-Gly-HMBA-polystyrene/1% divinylbenzene in deuterated DMF recorded with the LED sequence.[7] Sine-bell shaped gradients of 10 ms with increasing strengths (5, 25, 50, and 75% of a maximal 32 G/cm) were used in the spectra from top to bottom. Signals of solid-supported molecules (such as the amide protons at 7−9 ppm) do not lose intensity; whereas the signals of the soluble TMS (reference; 0 ppm) and the residual DMF (8.02 ppm) are decreasing.

First used in the context of protein NMR in aqueous solution,[6] the diffusion filter exploits the rapid diffusion of the water molecules, resulting in a rapid decay of its signal in a typical sequence used for diffusion measurements such as the LED sequence.[7] However, a major drawback of this sequence is that the nonnegligible diffusion of the protein also leads to severe losses in protein signal intensity. In the case of resin-bound moieties, however, the molecules retain reasonably free rotational diffusion, necessary to average out the anisotropic interactions such as homonuclear dipolar interactions and chemical shift anisotropy,[8] but lose completely their translational degrees of freedom due to the tethering to the solid framework. Therefore, in a typical diffusion experiment, the resonances of the attached molecules do not change in intensity as a function of gradient strength, whereas the NMR signal of molecules that freely diffuse does dephase and hence decays (Figure 1). A clear example is the NMR signal of the internal reference TMS (at 0 ppm) and the solvent signal of DMF at 8.02 ppm, which decrease in intensity upon increasing the force of the applied field gradient.

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(1) A useful bibliography in the field of combinatorial chemistry can be found at http://www.5z.com/divinfo/, the Web site of the journal *Molecular Diversity*, edited by Dr. M. Lebl.

(2) Yan, B. *Acc. Chem. Res.* **1998**, *31*, 621−630.

(3) (a) Anderson, R. C.; Stokes, J. P.; Shapiro, M. J. *Tetrahedron Lett.* **1995**, *36*, 5311−5314. (b) Anderson, R. C.; Jarema, M. A.; Shapiro, M. J.; Stokes, J. P.; Zilix, M. J. *J. Org. Chem.* **1995**, *60*, 2650−2651. (c) Fitch, W. L.; Detre, G.; Holmes, C. P.; Shoorely, J. N.; Keifer, P. A. *J. Org. Chem.* **1994**, *59*, 7955−7956. (d) Keifer, P. A. *J. Org. Chem.* **1996**, *61*, 1558−1599. (e) Sarkar, S. K.; Garigipati, R. S.; Adams, J. L.; Keifer, P. A. *J. Am. Chem. Soc.* **1996**, *118*, 2305−2306. (f) Pop, I.; Dhalluin, C.; Depréz, B.; Melnyk, P.; Lippens, G.; Tartar, A. *Tetrahedron* **1996**, *52*, 12209−12222.

(4) Dhalluin, C.; Boutillon, C.; Tartar, A.; Lippens, G. *J. Am. Chem. Soc.* **1997**, *119*, 10494−10500.

(5) (a) Smallcombe, S. H. *J. Am. Chem. Soc.* **1993**, *115*, 4776−4785. (b) Smallcombe, S. H.; Patt, S. L.; Keifer, P. A. *J. Magn. Reson., Ser. A* **1995**, *117*, 295−303.

(6) Van Zijl, P. C.; Moonen, C. T. *J. Magn. Reson.* **1990**, *87*, 18.

(7) (a) Gibbs, S. J.; Johnson, C. S. *J. Magn. Reson.* **1991**, *93*, 395−402. (b) Altieri, A. S.; Hinton, D. P.; Byrd, R. A. *J. Am. Chem. Soc.* **1995**, *117*, 7566−7567. (c) Dingley, A. J.; Mackay, J. P.; Chapman, B. E.; Morris, M. B.; Kuchel, P. W.; Hambly, B. D.; King, G. F. *J. Biomol. NMR* **1995**, *6*, 321−328.

(8) Haeberlen, U. *Advances in Magnetic Resonance* (Supplement 1). *High-Resolution NMR in solids*; Academic Press: New York, 1976.

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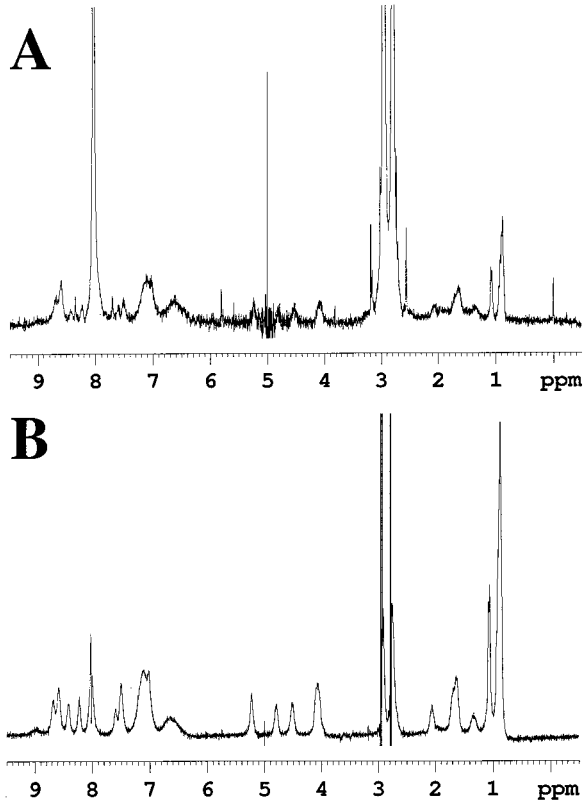

Figure 2. Proton spectra of 2.5 mg of Ile-Leu-Asn-Gly-HMBA-polystyrene/1% divinylbenzene in 90:10 DMF-h_7: DMF-d_7 (A) recorded with a single pulse experiment, (B) recorded with the LED diffusion sequence, using 8 ms duration and 24 G/cm for the defocusing gradients and 30 ms for the diffusion delay.

Figure 2A shows the 1D spectrum of a tetrapeptide anchored to a hydroxymethyl benzoic acid (HMBA) resin swollen in a mixture of 90% DMF-h_7 and 10% DMF-d_7 (the latter used as lock-signal). The intense solvent signal forces us to keep the receiver gain low, but the methyl resonances, with intensities comparable to the ^{13}C satellites of the DMF methyl groups, can clearly be distinguished. When we replace the single pulse experiment with the LED sequence, we completely suppress the solvent resonances and can effectively increase the gain to values comparable to those used for a resin in a deuterated solvent (Figure 2B). We do lose some signal intensity on the resonances of interest, mainly due to losses during the gradient pulse. The magnetization in the *xy* plane has indeed a larger line width than in solution, probably due to anisotropic magnetic susceptibility broadening that is not averaged to zero by the MAS.[9] However, the signal intensity remains perfectly acceptable even for more elaborate 2D sequences (see below), and in our experience, good results are obtained with gradient strengths greater than 20 G/cm during 8 ms, and with diffusion delays of the order of 30 ms.

The LED sequence element can successfully replace the first pulse in a TOCSY or HSQC experiment, and in Figure 3, we show the resulting 2D experiments on the same sample in deuterated (A) and protonated (B) solvents. Complete identification of the molecule is possible for both experimental conditions, without increase in time. Whereas these two sequences are the most useful for the assignment of molecular entities and therefore for the unambiguous identification of molecules in solid-phase synthesis, it might be of interest to look into structural aspects of resin-bound molecules by NOESY experiments. The LED sequence element cannot replace the first pulse of this sequence, as solvent resonances rapidly grow due to longitudinal relaxation during the mixing time. However, it is trivial to replace the last read pulse by the LED element, and a successful NOESY can be recorded in protonated solvent (Supporting Information).

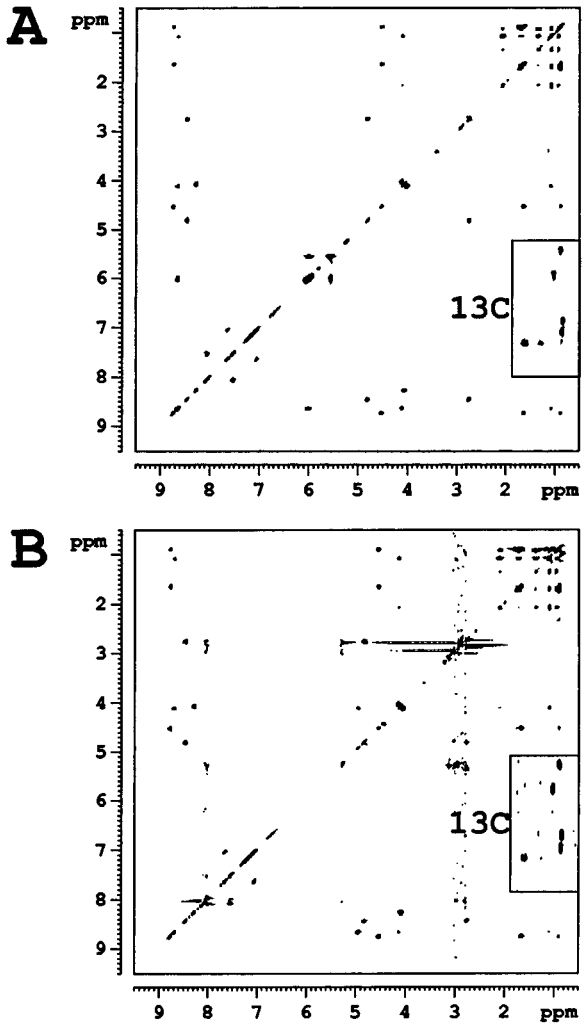

Figure 3. TOCSY spectra with a 80 ms mixing time of 2.5 mg of Ile-Leu-Asn-Gly-HMBA-polystyrene/1% divinylbenzene; (A) in DMF-d_7; (B) in 90:10 DMF-h_7:DMF-d_7. The insert shows the methyl regions of the corresponding HSQC spectra.

The solvent suppression scheme presented in this paper will have an important impact on the field of organic solid-phase synthesis as it completely reverses the relative difficulties experienced for the analysis of products coming from liquid or solid-phase synthesis. Indeed, solution analysis of organic molecules requires a high degree of purification and dissolution of the sample in a suitable deuterated solvent. The technique presented here, by taking of advantage of the differential diffusion of the tethered molecules, not only alleviates the requirement of using a deuterated solvent but also suppresses signals of the excess of reagents that have not reacted and of soluble byproducts of the synthesis. As was the case for the internal reference, TMS, these contaminants retain their translational degrees of freedom and will therefore be eliminated by the diffusion filter. Complete analysis of intermediates and monitoring of reactions are therefore expected to become straightforward in solid-phase synthesis, eliminating thereby one of the last obstacles for its wide application.

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JA9843728

(9) Elbayed, K.; Bourdonneau, M.; Furrer, J.; Richert, T.; Raya, J.; Hirschinger, J.; Piotto, M. *J. Magn. Reson.* **1998**, *136*, 127−129.