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High-Resolution ^{1}H NMR in Solid-Phase Organic Synthesis

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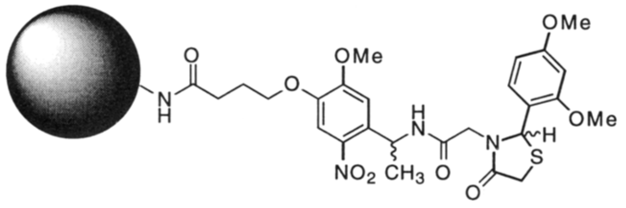
Summary: A method for obtaining ^{1}H NMR spectra of organic molecules covalently attached to solid-phase synthesis beads using magic angle spinning in a conventional spectrometer is described. The new method offers substantial qualitative and quantitative advantages and makes ^{1}H NMR truly available as an analytical tool to the solid-phase synthesis practitioner.

Merrifield"s solid-phase synthesis (SPS) concept[1] is widely used in biopolymer synthesis,[2] small molecule organic synthesis,[3] and recently, combinatorial chemistry.[4] As new chemistries continue to be developed on SPS, the need for new analytical tools for compound characterization has dramatically increased. A weakness of SPS, pointed out some time ago,[5] is that the chemist's favored tools for following a reaction, TLC and ^{1}H-NMR, are not applicable to the support-bound compounds. One is forced to rely on indirect methods, such as color tests (e.g., ninhydrin) or cleavage from the support, in order to assess the fate of a particular reaction. While ^{13}C NMR can be useful for characterizing the SPS end products,[6] it is often too slow for following reactions themselves.[7] We now report on the use of ^{1}H-NMR to characterize compounds *while still bound to the solid support.*

Proton NMR has sufficient sensitivity to allow it to be used for the rapid analysis of SPS reactions, but the typically large line widths of molecules bound to solids hinder data interpretation. As an example, Figure 1 (top) shows the conventional 500 MHz NMR spectrum of SPS product **1** which has been suspended in DMSO-d_6. Freely rotating molecules located in a homogeneous magnetic field should give narrow resonances in conventional high-resolution NMR spectroscopy. The broadening of the resonances shown in Figure 1 (top) could be due to either restricted molecular motion of compound **1** or inhomo-



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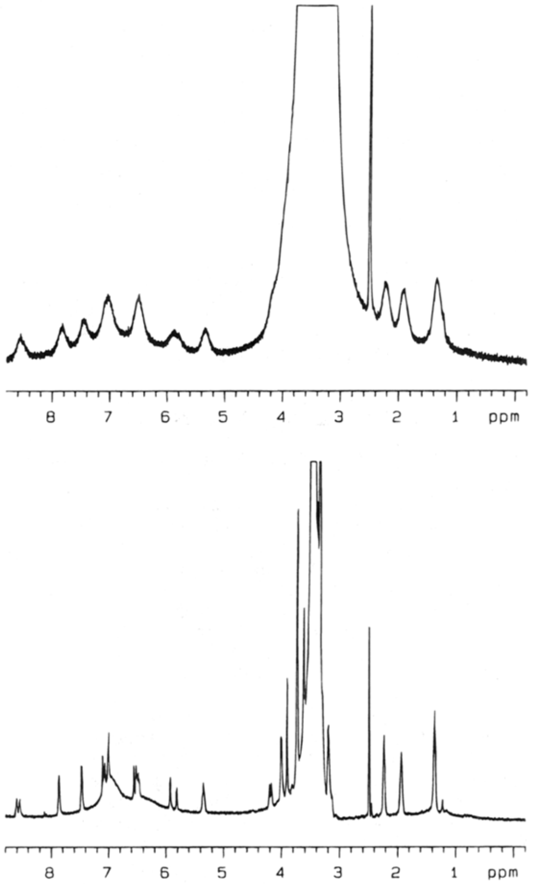

Figure 1. (Top) a 16-scan 500 MHz NMR spectrum of **1** (100 mg suspended in 600 μL of DMSO-d_6). The spectrum was obtained using a conventional 5 mm liquids probe. (Bottom) a 16-scan 500 MHz NMR spectrum of **1** (10 mg suspended in 30 μL of DMSO-d_6). The spectrum was obtained by spinning at 2000 Hz about the magic angle in a Varian Nano-NMR probe.

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geneities in the magnetic field immediately surrounding the bead. The latter arises from magnetic susceptibility discontinuities at the interface of the bead and the solvent; such an effect would certainly contribute to line broadening.

Magic-angle spinning (MAS) is used extensively in solid-state NMR to remove line broadening caused by chemical-shift anisotropy.[8] While MAS is typically applied to rigid solids, it has produced line narrowing in rubbery polymers such as polybutadiene[9] and solvent-swollen polystyrene gels[10] and proven useful for homogeneous solutions.[11] Recent work has shown that MAS is also a useful method for the removal of bulk magnetic-susceptibility discontinuities that cause the poor line shapes which are typically found in the spectra of very small liquid NMR samples.[12] We are finding that MAS is useful for obtaining high-resolution spectra for any liquid-phase sample, present as either a solution or a suspension, which might otherwise have unacceptably broad line shapes due to susceptibility discontinuities. We demonstrate here the use of a Varian Nano-NMR probe, which has been specifically designed to exploit MAS for very small ($<40\ \mu$L) liquid samples, for obtaining narrow-line ^{1}H NMR spectra of molecules attached to SPS beads.[13]

Test compound **1**[14] was synthesized on TentaGel beads.[15] The resultant beads (10 mg, containing about 3 μmol or 1.5 mg of molecule) were suspended in 30 μL of DMSO-d_6 and transferred to a Nano-NMR probe cell. Sixteen transients were sufficient to yield the ^{1}H spectrum shown as Figure 1 (bottom). The broad peaks at 6–8 ppm are from the immobile polystyrene matrix. The large resonances between 3–4 ppm are due to H_2O and the more solvent accessible poly(ethylene glycol) side chains of the matrix. The remaining peaks are readily assigned to the protons of **1**.

It is instructive to compare in detail the conditions for these two spectra. The lower MAS spectrum of Figure 1 shows a 3-fold improvement in signal to noise ratio using one-tenth the sample relative to the top spectrum of Figure 1. But more important than this quantitative improvement is the qualitative improvement in the information content of the bottom spectrum in Figure 1 over the top spectrum (e.g., the observation of the multiple signals from 3.6–4.2 ppm). The individual resonances are readily assigned to **1**, and one truly has a sense of what compound(s) is (are) present on the support. Thus, the recent advances in MAS now make ^{1}H-NMR a practical analytical tool for SPS.

A significant advantage of this NMR protocol is that it is nondestructive as compared to the traditional "cleave and characterize" techniques in use today for SPS. The application of MAS to SPS makes it unnecessary to cleave the molecule from the bead. One can now follow a reaction spectroscopically and fully ascertain the fate of a compound without fear of side reactions (racemization, isomerization, etc.) which may be irrelevant to the molecular assemblage present on the solid phase.

This NMR method should prove useful for following reactions, identifying SPS products (and impurities), and strengthening the foundation for the continued development of SPS in organic chemistry. Standard 2D NMR and solvent suppression pulse sequences are also routine with this probe and may be exploited as we explore further the applications of MAS NMR to organic chemistry on solid supports.

Supplementary Material Available: The synthetic procedure for **1**, including an assignment of the peaks in Figure 1 (bottom) and the NMR spectrum of underivatized TentaGel beads (4 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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(14) The solid-phase synthesis of 4-thiazolidinones and the development of new photocleavable linkers will be reported in due course (Holmes, C. P. Unpublished Results).

(15) TentaGel S NH_2 (Rapp Polymere) with particle size 90 μm and nominal loading of 300 μmol/g was used for this experiment. TentaGel is a copolymer of cross-linked polystyrene and poly(ethylene glycol) in a w/w ratio of roughly 1:3.