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Analytical Techniques in Combinatorial Chemistry: MAS CH Correlation in Solvent-Swollen Resin

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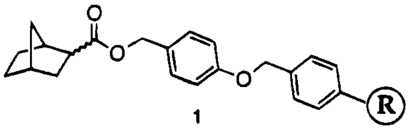
Recent advances in combinatorial chemistry[1-3] have revived the chemistry community's interest in solid phase synthesis, particularly of nonpeptidic molecules. Polymer-based solid phase synthesis is amenable to many of the synthetic techniques normally associated with solution chemistry, allowing its application to be extended beyond the more traditional areas of peptide and nucleic acid synthesis to the synthesis of more structurally diverse molecules.[4] Multiple, simultaneous syntheses of templates such as hydantoins[2] and benzodiazepines,[2,3] or β-mercapto ketones[5] are recent demonstrations of the enormous potential of polymer-supported chemistry in generating libraries of small organic molecules.

An important potential drawback of multistep, solid phase synthesis is the paucity of rapid, readily available, nondestructive analytical techniques. During the course of such syntheses and particularly for the optimization of reactions, it would be of great benefit to characterize reaction products of sequential transformations in order to monitor potential synthetic problems such as unexpected isomerization or side-product formation. Gel phase ^{13}C NMR[6] and "on-bead" FT-IR have proven useful in determining the success or failure of some solid phase, chemical transformations.[2,5,7] A recent report concering the utilization of a Varian "Nano-NMR probe" demonstrated the utility of magic-angle spinning (MAS) for line narrowing in ^{1}H NMR of TentaGel-bound substates.[8]

We demonstrate here that MAS ^{13}C–^{1}H correlated NMR of organic molecules attached to solvent-swollen polymers with a standard solid state probe is possible. This technique promises to be useful not only in the determination of the success or failure of a chemical transformation but in the structural verification of molecules produced by combinatorial reactions, as well as detection of diastereomers and side products.

Several factors influenced the design of our experiments: (1) Because signals due to the aromatic functionality of the polymer would complicate interpretation of data in this region of the ^{13}C spectrum, we wanted to consider a nonaromatic molecule only. (2) While ^{13}C gel phase NMR data are useful, they invariably suffer from broad resonances that result in inadequate resolution and loss of information, particularly for functional groups such as carbonyls. However, MAS ^{13}C NMR data from solvent-swollen beads provide well-resolved resonances,[9] and it was hoped that carbonyl signals would be detected using this technique. (3) We wished to simulate a solid phase reaction complication where two subtly different products could result. (4) The effect that linker length would have on the resolution of the resonances of the molecule was unknown. In order to address all of these issues, we chose to prepare samples of norbornane-2-carboxylic acid, as a mixture of epimers,[10] attached to polystyrene resin with linkers of varying lengths.



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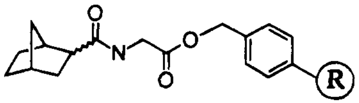

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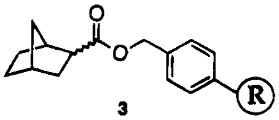

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Benzene was the solvent of choice for these experiments not only because of its known ability to effectively swell resins[11] but because its ^{13}C NMR signal overlaps with those of the polymer aromatic functionality, thereby circumventing the problem of additional "impurity" peaks.

Shown in Figure 1 is the MAS ^{13}C solid state NMR spectrum of sample **1**, with the gel phase ^{13}C spectrum given for comparison. The MAS spectrum clearly shows

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(6) Gel phase NMR is a term that represents insoluble polymer-bound organic substrate NMR. A **slurry** of the material in benzene or appropiate solvent is placed in the NMR tube, and spectra are obtained with typical solution NMR conditions.

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(9) ^{13}C and ^{1}H NMR spectra were obtained on a Bruker AMX-400WB spectrometer operating at 100.627 MHz for ^{13}C and 400.13 for ^{1}H equipped with a 7 mm MAS probe. The ^{13}C and heteronuclear correlation spectra were obtained using the standard high-resolution pulse sequence. The final data set was 2K by 140, zero filled to 512. 512 transients were recorded for each of the 140 increments. To the sample (75 mg, dry weight) was added enough benzene-d_6 to swell the beads. The **solid granulated** resin (as opposed to a slurry for gel phase NMR) was placed into a standard 7 mm rotor. MAS spectra were obtained at a rotor speed of 5 kHz. Gel phase and solution NMR data were obtained on a Bruker AM-500 spectrometer operating at 125.62 MHz for ^{13}C and 500.13 MHz for ^{1}H, equipped with a 5 mm dual C/H probe.

(10) Norbornane-2-carboxylic acid, purchased from Aldrich Chemicals, exists as a 70/30 exo/endo mixture of epimers as determined by ^{13}C NMR and confirmed by HPLC analysis.

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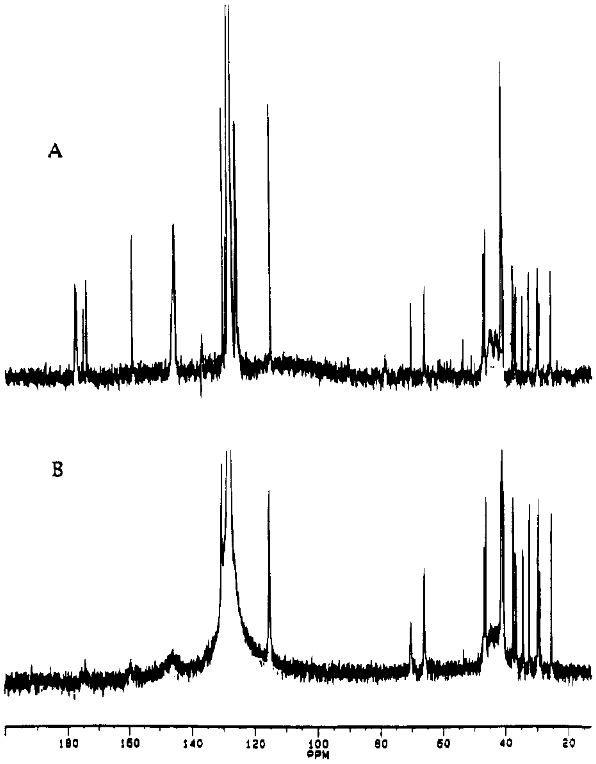

Figure 1. ^{13}C NMR spectrum of **1**. (A) MAS of benzene-swelled resin. (B) Gel phase.

carbonyl resonances (δ = 174.9, 173.8 ppm), as well as signals due to the polystyrene bead carbon atoms. The analytical advantage of obtaining a solution quality carbon-13 NMR spectrum is obvious. Whereas commercially available norbornane-2-carboxylic acid exists as a 70:30 *exo*/*endo* mixture of epimers, the *exo*/*endo* ratio of resin-coupled norbornane-2-carboxylic acid was semiquantitatively determined to be 60:40, based on the peak intensities in the MAS ^{13}C spectrum. This ratio was subsequently confirmed by GC analysis of the acid mixture obtained after cleaveage from the resin. Regardless of the origin of the epimer ratio perturbation, the fact that such a change can be detected is not only remarkable but also illustrative of the superior signal resolution provided by MAS ^{13}C spectroscopy.

The MAS ^{13}C spectrum of the solvent-swollen resin exposes sharp signals for all of the carbons in the norbornyl system, with an average carbon resolution of 13 Hz. The high motional freedom exhibited by the molecules on the swollen bead and hence solution-like properties results in sharp resonances of tether carbon atoms as well (δ = 158.8, 114.6, 69.7, 65.4 ppm).[12] The observation of the tether carbons is extemely important, providing a straightforward means of determining the extent of reaction since an internal reference is observed in the resonances of the tether. Similar NMR results were obtained for samples **2** and **3**, indicating that the length of the linker is not a factor in the resolution of the MAS ^{13}C spectrum.

The signals in the MAS proton NMR spectrum of sample **1** have a line width of only 26 Hz and are of similar quality to that obtained in the "Nano" probe. Although this line width could be considered broad for high-resolution studies, for MAS ^{1}H NMR, where proton line widths on solid materials can be measured in kHz, it is relatively sharp and allows structural information to be obtained by evaluation of CH-correlated data. The MAS CH-correlated spectrum of sample **1** and the solution state spectrum of norbornane-2-carboxylic acid, for comparison, are shown in Figure 2. The carbon and proton resonance assignments follow in a standard fasion directly from consideration of chemical shifts and proton multiplicity.

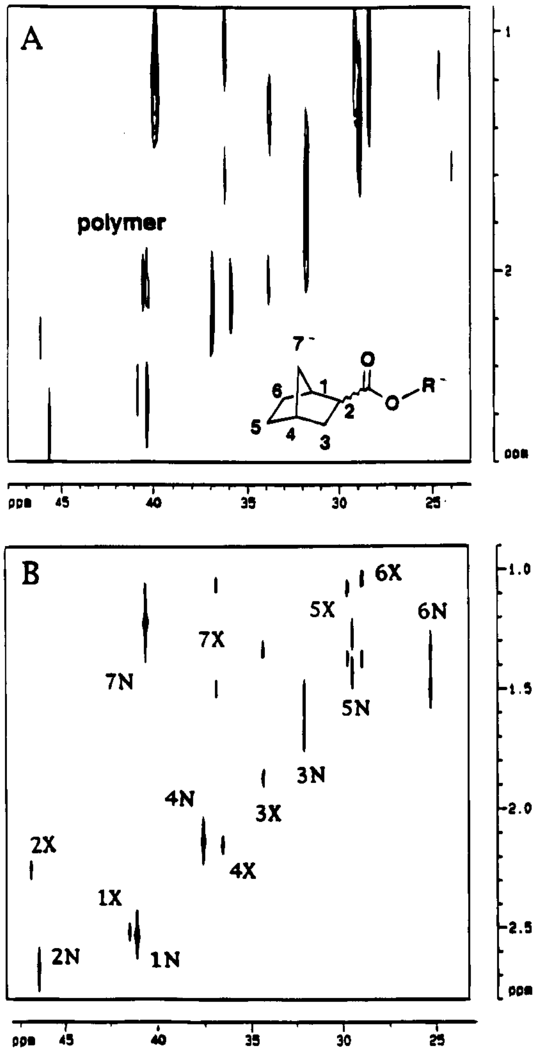

Figure 2. CH-correlated NMR spectrum of **1**. (A) MAS of benzene-swelled resin. (B) Solution CH-correlated spectrum for a mixture of epimers of norbornane-2-carboxylic acid. Assignments are indicated for endo (N) and exo (X) epimers.

As can be seen from these spectra, the correspondence between the MAS data of norbornane-2-carboxylic acid on resin and the spectrum of norbornane-2-carboxylic acid in solution in a non-MAS probe is excellent, and resonance assignment and structure verification can be accomplished. We believe that this demonstration of the ability to obtain high-resolution liquid quality ^{13}C and CH-correlated NMR data and, in principle, other data such as HMBC, COSY, and NOESY[13] on the solvent-swollen solid using only MAS will be of great utility, enhancing the development of combinatorial chemistry.

Acknowledgment. We thank Jefferson Chin for obtaining 500 MHz ^{1}H NMR data.

Supplementary Material Available: Experimental procedures and proton NMR for **1** (3 pages).

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