A Case Study of Employing Spectroscopic Tools for Monitoring Reactions in the Developmental Stage of a Combinatorial Chemistry Library

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Received February 20, 1998

Organic synthesis on solid-phase resins requires new applications of nondestructive analytical tools to monitor the progress of reactions. Herein we describe our novel approach to validate a five-step reaction sequence on resin. We have developed a protocol list that allows us to follow reactions step-by-step using different analytical procedures in the order of increasing experiment time. Results from these various techniques for each reaction step are contrasted and compared. The key to achieve definitive analytical data for the entire reaction sequence is the combined application of more than one analytical technique.

Introduction

Technological advances in the past few years have led to vast changes in the drug discovery paradigm employed by the pharmaceutical industry. One facet of this dramatic change has occurred with the use of combinatorial chemistry on solid-phase supports or resins.[1] This formidable technique by which one can synthesize large numbers of compounds very rapidly in concert with the emergence of high-throughput screening allows for the rapid determination of new leads for a number of targets.[2] The lack of powerful analytical techniques for monitoring on-resin reactions has become a major hindrance to using new chemistries on resin. More specifically, a large impedance to this process is the determination of the range of reagents which give the desired intermediates or compounds in adequate yields and the proper conditions necessary to optimize reaction conditions. In most cases, much time and large amounts of resin must be devoted to using the synthesis/cleavage routine. Cleaved intermediates and products from beads can then be analyzed by traditional methods such as MS, HPLC, NMR, and IR. However, many intermediates are not stable under cleavage conditions and cannot be isolated. Early efforts to avoid this problem were demonstrated by the strategic use of ^{13}C-enriched building blocks and ^{13}C NMR.[3,4] Others have employed ^{19}F and ^{15}N among other NMR techniques.[5−7] However, as the chemistries become more sophisticated, the use of specially labeled building blocks may not be an option because of availability or cost. More recently, it has been reported that nondestructive on-bead detection of organic compounds by MAS (magic angle spinning), NMR,[8−10] or FTIR[11−15] can be a useful alternative. Herein we disclose our results on the application of single-bead FTIR and, concurrently, MAS NMR for the nondestructive monitoring of a reaction sequence from initial loading to generation of the desired product. The stepwise sequence in the synthesis of the desired compounds (Scheme 1) is derived from an actual production library of trisubstituted amines.[16] The intermediates that are not acid cleavable from the hydroxymethyl polystyrene resin[17] can be characterized only by on-resin analytical techniques. Traditional analytical methods are only capable of characterizing the final product but do not allow for monitoring the progress of reactions.

Results and Discussion

Single-bead FTIR (Figure 1A) and ^{1}H (Figure 2A) and ^{13}C (Figure 3A) MAS NMR spectra of hydroxymethyl polystyrene resin **1** were taken as a baseline observation. The formation of acrylate **2** by reacting hydroxymethyl resin **1** with acryloyl chloride was indicated by the appearance of carbonyl stretching at 1725 cm^{-1} and

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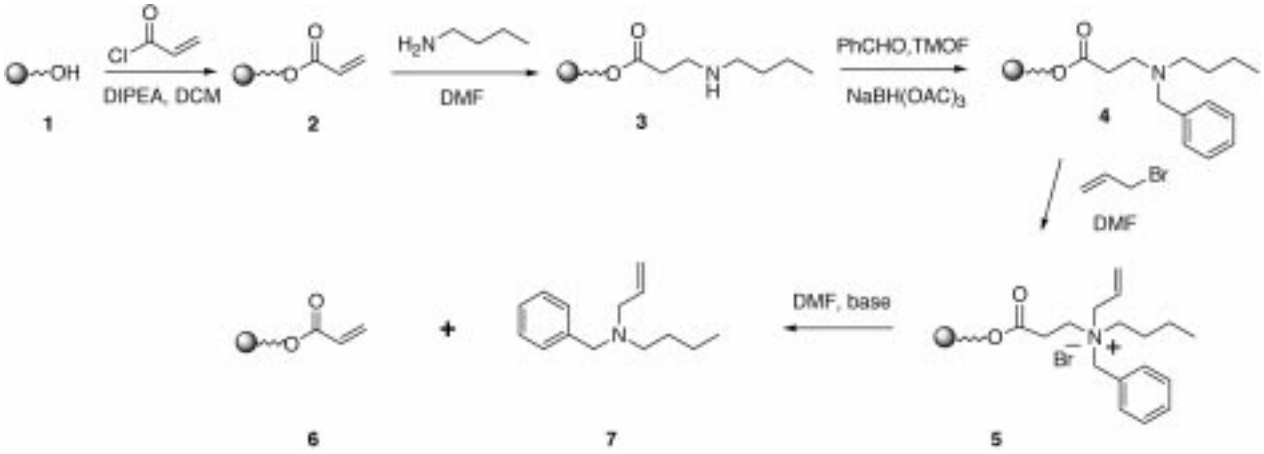
(17) Resin purchased and used as is from Novabiochem.

10.1021/jo980322+ CCC: $15.00
Published on Web 11/10/1998

Scheme 1: Solid-Phase Synthesis of a Trisubstituted Amine



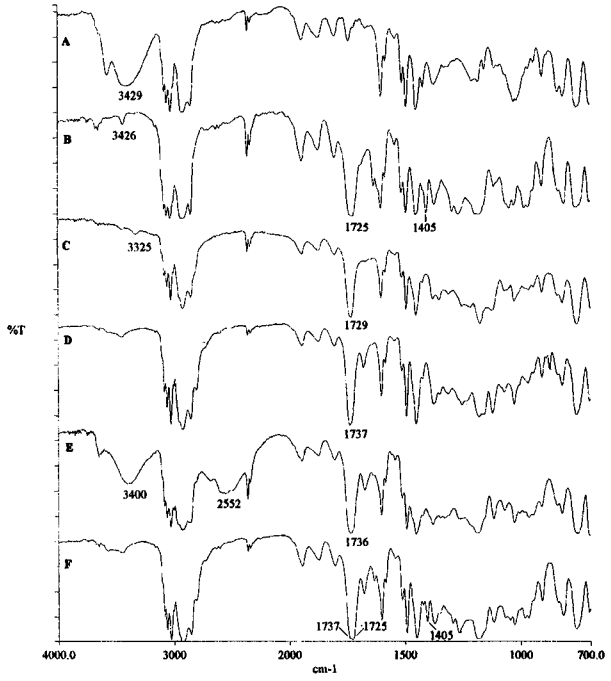


Figure 1. Single-bead FTIR spectra of resins **1** (A), **2** (B), **3** (C), **4** (D), **5** (E), and **6** (F).

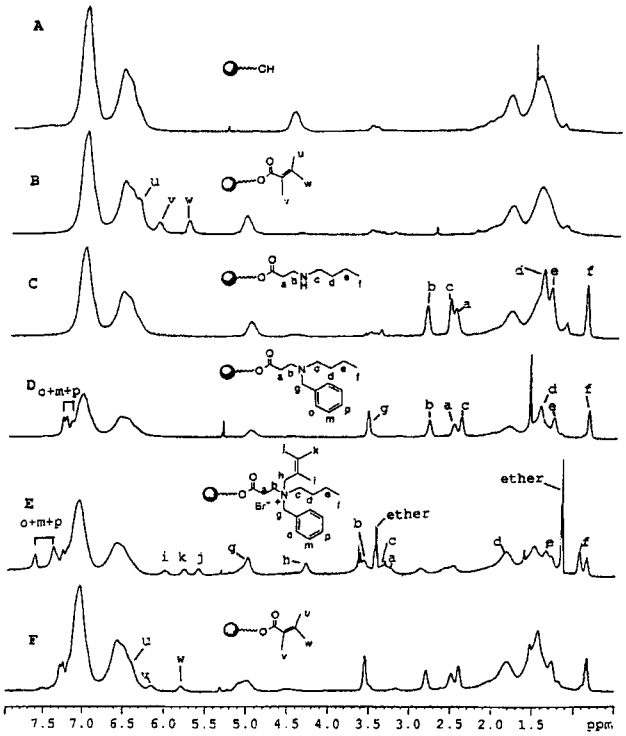


Figure 2. ^{1}H MAS NMR spectra of resins **1** (A), **2** (B), **3** (C), **4** (D), **5** (E), and **6** (F). Diethyl ether is a wash solvent during the washing stage.

alkene C−H in-plane bending at 1405 cm^{-1} in the IR spectrum in Figure 1B. The reaction nearly achieved completion on the basis of the residual O−H stretching frequency. The ^{1}H NMR spectrum in Figure 2B revealed three vinyl proton resonances in which two protons at 5.8 and 6.4 ppm were well separated and a third proton resonance at 6.8 ppm was on the shoulder of the polystyrene background. The ^{13}C NMR spectrum in Figure 3B confirmed the identity of acrylate **2** by the appearance of a carbonyl group at 166.4 ppm and vinyl carbons at 131.2 and 129.0 ppm. The Michael addition was observed by the disappearance of the alkene C−H in-plane bending absorption and the appearance of a weak N−H stretching at 3325 cm^{-1} as seen in the IR spectrum in Figure 1C. The carbonyl stretching shifted to a higher frequency because of the loss of conjugation. ^{1}H NMR characterized this reaction step well in that the vinyl protons disappeared completely and six new aliphatic proton peaks appeared in the 0.9−2.8 ppm range of Figure 2C. The ^{13}C NMR spectrum in Figure 3C showed a downfield shift of the carbonyl peak to 173.1 ppm, the disappearance of the vinyl carbons, and the appearance of six distinct aliphatic resonances in the 14.5−50.0 ppm range. Although not conclusive, the IR spectrum in Figure 1D indicated the presence of the trisubstituted amine **4** by the shift of the carbonyl group to a higher frequency with the addition of an electron-withdrawing benzyl group. The ^{1}H NMR spectrum in Figure 2D of amine **4** was well characterized by the presence of three aromatic protons and benzylic methylene protons at 3.60 ppm. The ^{13}C NMR spectrum in Figure 3D shows two benzylic aromatic carbon resonances at 140.1 and 129.3 ppm outside the broad resin peak and the benzylic methylene carbon resonance at 60.0 ppm. IR analysis of reaction II and NMR analysis of reaction III show the reactions are complete.

In the next step, the tetrasubstituted amine salt **5** was formed by alkylation with allylic bromide. The IR spectrum in Figure 1E does not give an indication that

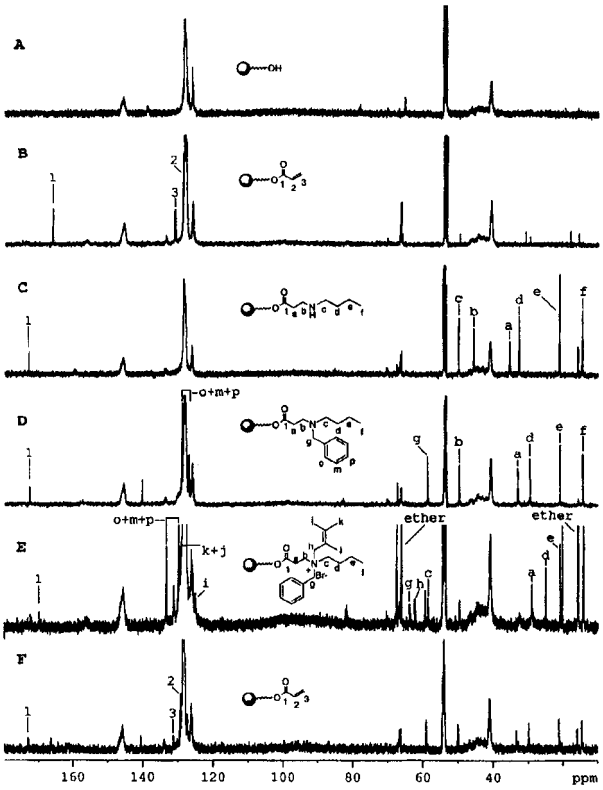


Figure 3. ^{13}C MAS NMR spectra of resins **1** (A), **2** (B), **3** (C), **4** (D), **5** (E), and **6** (F).

the alkylation occurred. The 2552 cm^{-1} absorption is likely due to the tertiary amine salt[18] that is formed by protonating the amine **4** by a trace amount of acid in allylic bromide. The ^1H NMR spectrum in Figure 2E shows the three aromatic proton peaks in the range of 7.4−7.6 ppm shifted outside the broad polystyrene peak and the diagnostic allylic proton peaks in the range of 4.3−6.0 ppm. However, in the aliphatic region of 1.3−3.7 ppm, the peaks overlap and are difficult to interpret. On the basis of the 2D MAS COSY[19] spectrum,[20] not only were the remaining six aliphatic protons from the salt **5** assigned but also the aliphatic protons from trisubstituted amine **4** were identified, which indicates incomplete quaternization. Because of the protonation of the amine, the chemical shifts of amine **4** were shifted slightly compared to those in Figure 2D. A 59 ± 5% conversion was estimated on the basis of the methyl intensities as seen in the ^1H NMR spectrum in Figure 2E. The reaction was iterated with longer reaction times, and the percent conversion was improved to 77 ± 5%.[20] In the final step of the reaction sequence, acrylate **6** was regenerated by Hofmann elimination of the quaternary salt **5** while providing product **7**. The IR spectrum in Figure 1F exhibited the regenerated acrylate by a shift of the carbonyl group to a lower frequency and the re-appearance of alkene C−H in-plane bending at 1405 cm^{-1}. However, the IR spectrum in Figure 1F looks more like that in Figure 1D, which indicates the presence of tri-

Table 1. A Comparison of the Analytical Techniques on Monitoring Reactions in Scheme 1[a]

increasing experiment time ↓

Techniques	reaction I	reaction II	reaction III	reaction IV	reaction V
single bead IR	**1**	**1**	3	4	3
^1H MAS NMR	1	1	**1**	**2**	**1**
MAS COSY	1	1	2	2	1
MAS HMQC	1	1	1	1	1
^{13}C MAS NMR	1	1	2	2	1

[a] The outlined region indicates the most efficient route to monitor the five-step reaction sequence. 1 = entirely observable and conclusive; 2 = partially observable and conclusive; 3 = partially observable and inconclusive; 4 = not observable and inconclusive.

substituted amine **4** as a result of incomplete quaternization from reaction IV. The ^1H NMR spectrum in Figure 2F shows the three vinyl proton resonances from the acrylate and the characteristic aromatic and aliphatic resonances of trisubstituted amine **4**. Additionally, ^1H NMR indicates that the Hofmann elimination was complete by the absence of the tetrasubstituted ammonium salt **5**. The ^{13}C NMR spectrum in Figure 3F also demonstrates the regeneration of the acrylate by its carbonyl and vinyl carbons. The overlapping of the spectra in Figure 3 reveals the presence of trisubstituted amine **4** resonances and the absence of tetrasubstituted ammonium salt **5** resonances in the regenerated acrylate resin as detected in the ^1H NMR discussed previously. Examination of the regenerated acrylate **6** by both IR and NMR (Figures 1F, 2F, and 3F) indicates the product **7** was not left on the beads. The final product **7** was isolated and evaluated by traditional analytical methods.[16] All of the ^1H and ^{13}C NMR assignments were confirmed by 2D MAS COSY and HMQC[21] experiments.[20]

Table 1 gives a qualitative view of the effectiveness of the analytical techniques used to monitor the five consecutive reactions in Scheme 1. The techniques are listed in order of increasing experiment time. Single-bead FTIR is the first choice of the five analytical techniques based upon sensitivity, convenience, and effectiveness in monitoring reactions that involve functional group transformations with diagnostic IR adsorptions. Single-bead FTIR requires no special preparation of resin samples and provides rapid turnaround time. In addition, the extent of disappearance of an absorption band allows for calculation of a percent conversion to product for a reaction. However, when no suitable functional group appears or a group disappears, one can predict that FTIR is not diagnostic as seen in reactions III and IV. In such cases, we relied upon ^1H MAS NMR to conclusively identify the products. The use of MAS requires special sample preparation, a special probe, and possible probe change, increasing the complexity of the technique and experiment time. As demonstrated in Figure 2A−F, ^1H MAS NMR is effective in monitoring all five reactions in the reaction scheme. For reaction IV, the progress of the reaction was monitored by the key ^1H NMR signals (allylic) without complete assignment of amine **5**. When the peaks overlapped and became too ambiguous to assign in ^1H NMR, 2D MAS COSY became the next option because of its shorter experiment time when

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compared to that of ^{13}C MAS NMR and HMQC. ^{13}C MAS NMR and HMQC did not demonstrate any advantage over COSY in our reaction sequence but did provide further proof of desired compounds. With the relatively narrow line widths and large dispersion of chemical shifts in the ^{13}C NMR dimension, we anticipate HMQC and/or ^{13}C MAS NMR to be very useful when analyzing more complex structures. The most efficient way of monitoring the entire reaction scheme is to follow the techniques indicated in the outlined region of Table 1. Although organic reactions may vary depending on the synthetic routes, this protocol creates a general approach to monitoring reactions on resins.

Summary

The products in each reaction step of the combinatorial library were analyzed on resin using five analytical techniques in order to evaluate their importance in monitoring a reaction series. These techniques included single-bead FTIR, ^1H MAS NMR, 2D MAS COSY, MAS HMQC, and ^{13}C MAS NMR. We next ranked the effort based on time required to run the experiments. Despite the advantages of single-bead FTIR, one can predict its failure to monitor reactions which have no changes in functional groups with characteristic IR absorptions. For the reactions discussed above, the combination of IR and ^1H MAS NMR is more efficient than IR alone in evaluating the reactions in the development phase of this library. In addition, we have also shown the power of MAS COSY, MAS HMQC, and ^{13}C NMR for more complicated problems. *The true power of these techniques is best exploited when used in combination.* Our data generated a protocol, in rank order, of analytical tools that allows a chemist to decisively evaluate synthetic steps, verify new building blocks, and detect possible side reactions prior to or during actual library construction.

Experimental Section

FTIR Microscope. A Perkin-Elmer i-series FTIR microscope equipped with a liquid-nitrogen-cooled mercury−cadmium−telluride (MCT) detector and the IMAGE system interfaced with a Spectrum 2000 FTIR spectrometer were used to acquire IR spectra of beads. AutoImage and Spectrum 2000 software were used to acquire and process data. A transmission mode and an aperture size of 100 mm × 100 mm were used for measurement. The spectra were scanned from 4000 to 700 cm^{-1} and averaged over four scans. A flattened resin bead was placed directly on a BaF_2 disk used for IR detection. The background spectrum was collected at a blank spot on the same BaF_2 window with resolution of 8 cm^{-1}. To ensure one sampling point was representative of a mass of samples, multiple beads were sampled. There was no significant difference in IR spectra.

High-Resolution MAS NMR. Solid-phase HR MAS (high-resolution magic angle spinning) NMR spectra were obtained on a Bruker DRX 500 spectrometer using a 4 mm HR MAS probe. Hydroxymethyl resin (5 mg) was preswollen in deuterated dichloromethane and spun at 3.5 kHz about the magic angle (54.7°). TMS was used as the internal reference. Only eight scans were collected for each proton experiment. The total experiment time for proton-decoupled ^{13}C NMR was 4 h (7000 scans, 0.9 s acquisition time, and 1.0 s relaxation delay). 2D COSY spectra were acquired in 36 min using 16 scans for each $128t_1$ increment with a relaxation delay of 2 s. The ^1H and ^{13}C HMQC spectra were obtained with a BIRD sequence in 140 m (16 scans for each $220t_1$ increment, 0.085 s acquisition time, and 1.5 s relaxation delay).

Supporting Information Available: A confirmation of assignments for resins **1**−**6** including 2D COSY and HMQC spectra (16 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.

JO980322+