Infrared Spectrum of a Single Resin Bead for Real-Time Monitoring of Solid-Phase Reactions

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Two applications of combinatorial chemistry,[1-3] lead-finding and chemical analoging, are likely to rely on solid-phase synthesis to facilitate the assembly of combinatorial libraries of compounds. Because of the inadequate pharmaceutical properties of peptides and nucleotides, intense effort has been on the generation of small organic compound libraries.[4-6] A major hindrance to this work has been the lack of analytical methods to establish the degree to which the expected chemistry has proceeded on polymer supports. Even more difficulties are encountered for monitoring solid-phase reactions during the course of a reaction (real-time monitoring). We report here a novel single-bead FTIR microspectroscopy method for real-time monitoring of organic synthesis on resin support. We describe (1) the determination of the extent of conversion from starting materials to products, (2) the ability of a single bead to be representative of a mass of beads, and (3) the monitoring of the time course of a reaction.

FTIR spectra of reaction products on beads have been reported by a method which requires making a KBr pellet with >10 mg of beads.[7,8] Using FTIR microspectroscopy, we obtained spectra with a high signal/noise ratio (Figure 1) on a single resin bead in less than 5 min. We took a drop of resin suspension during a reaction and obtained an IR spectrum of a bead after a quick wash and dry. In this way, we easily obtained a reaction time course which would be hard to obtain using a KBr pellet method, considering the small scale of the solid-phase synthesis. A bead with a diameter of 80–100 μm typically carries about 400–800 pmol of attached compound. The smallest bead to give high-quality spectra using FTIR microspectroscopy was Merrifield resin of 50 μm diameter. In this case, a single bead containing 100 pmol of attached compound represents the lowest detection limit of this technique currently achieved by us.

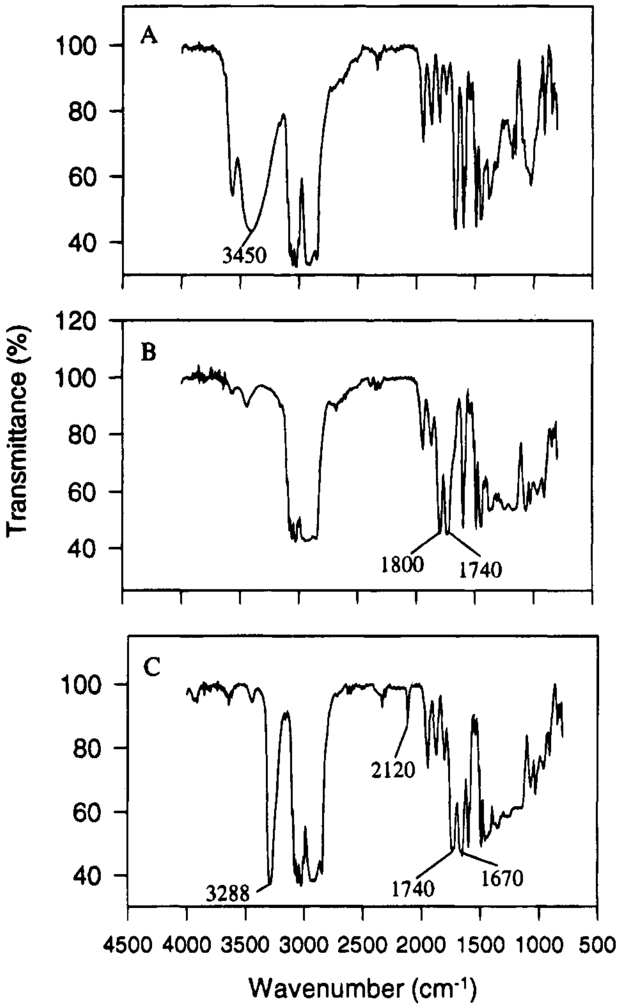

Figure 1. Reaction products monitored by single-bead IR. Reaction II was carried out on the hydroxymethyl resin. IR spectrum of the starting hydroxymethyl resin is shown in A. After 12 h of reaction, compound **1** was converted to compound **2** as shown in B. In the next step (C), the acid chloride was converted into **3**. The spectrum obtained after 18 h of reaction clearly indicated the desired conversion.

A sequence of synthetic reactions on hydroxymethyl polystyrene resin (Scheme 1, reaction I) was monitored. At 12 and 18 h after synthetic steps 1 and 2, respectively, a drop of resin suspension was removed from the reaction vessel, washed with solvent, and dried under vacuum. Single-bead IR spectra were then recorded. A >95% conversion of the starting material **1** (Figure 1A) to the intermediate **2** (Figure 1B) was estimated by observing the disappearance of the OH stretch vibration at 3450 cm^{-1} and the emergence of the acid chloride carbonyl at 1800 cm^{-1} and the ester carbonyl at 1740 cm^{-1}. The transformation from **2** to **3** was unambiguously detected by the formation of two distinct bands, the carbon–carbon triple bond stretch at 2120 cm^{-1} and the stretch of C–H from the terminal alkyne at 3288 cm^{-1} shown in Figure 1C. A >95% conversion was estimated on the basis of the nearly complete shift of the acid chloride carbonyl stretch from 1800 cm^{-1} to an amide carbonyl stretch at 1670 cm^{-1}.

The speed and the sensitivity of FTIR microspectroscopy provide an avenue for real-time monitoring of synthetic reactions on resin supports. However, one cannot use a single resin bead as a probe for reactions on a mass of beads if it does not represent the entire population of the reaction mixture. To investigate the homogeneity of the reaction on beads, we selected four

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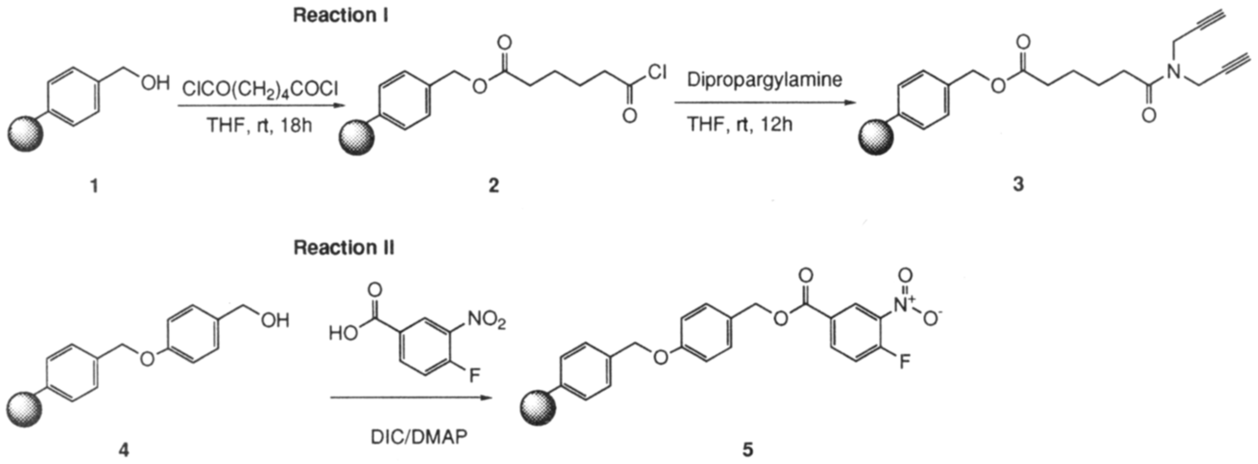
Scheme 1



samples representing hydroxymethyl, Wang, NovaSyn TGA, and Rink amide resins. We recorded single-bead spectra of 30 randomly chosen beads from each sample. The results from two of these studies are described here. Compound **2** in reaction I was quantitated by measuring its absorbance (1740 cm^{-1}) relative to the starting material (a resin peak at 1945 cm^{-1}) for all 30 beads. The quantity of compound **5** in reaction II was also measured by its absorbance (1722 cm^{-1}) relative to the starting material (1649 cm^{-1}). For each of these two examples, as well as other examples on NovaSyn TGA and Rink amide resins, the amount of synthetic product was determined to be identical within experimental error (±5%) for all beads examined using the intensity ratio of the absorption bands as an indicator of compound on the bead. These results demonstrate that, for these reactions, a single bead is indeed representative of the entire population of beads in the reactor.

There are few convenient methods for analyzing the progress of solid-phase reactions. The single-bead FTIR technique offers a novel tool for monitoring the time course of a reaction on resin support. We collected an IR spectrum of a single bead at various time intervals after the initiation of the reaction II. The time dependence of IR intensity of the ester carbonyl stretch at 1722 cm^{-1} and the nitro group stretch at 1540 cm^{-1} relative to resin intensity at 1943 cm^{-1} was monitored and is shown in Figure 2. This result indicates that this particular reaction reached steady state within 20 min. With this technique in hand, an understanding of reaction time course on various solid supports becomes achievable.

FTIR microspectroscopy has been used for analyzing microcontaminants, polymers, and semiconductors for decades.[9] In this study, we have applied this technique for the first time to the determination of the product identity, reaction homogeneity, and reaction time course on solid supports by analyzing a single resin bead. This method is potentially important in characterizing a compound from a "split-and-pool" library in which the content of a single bead is homogeneous. Proper selection of linkers on resins may further extend the scope of applicable reactions.[10] This technique provides a powerful analytical tool for solid-phase synthesis, and we are currently applying this new method to the characterization of combinatorial libraries.

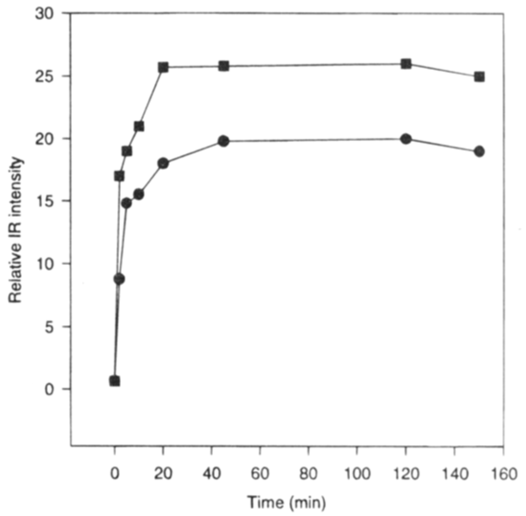


Figure 2. Reaction progress on resin support. The FTIR spectra of products on Wang resin from reaction II were recorded at room temperature. The formation of compound **5** represented by relative intensities of the product and starting material bands: 1722/1943 for the carbonyl (circles) and 1540/1943 for the nitro group (squares) were plotted against time.

Experimental Section

Materials and Sample Preparation. Hydroxymethyl resin was purchased from Advanced ChemTech, Wang resin from Midwest Bio-Tech, NovaSyn TGA resin from Novabiochem, Rink amide resin from DuPont, and Merrifield resin from Aldrich. A drop of resin suspension was taken out of the reaction vessel at specified times. The resins were filtered, three times with THF (reaction I) or DMF (reaction II) and then with methanol. The sample was dried under vacuum (0.5 mmHg) for 30 min.

FTIR Microspectroscopy. All spectra were collected on a BIO-RAD FTS-40 spectrophotometer, using an SPC-3200 data station. The optical bench is coupled with a UMA-300 IR microscope. The microscope is equipped with a 36X Cassegrain objective and a liquid nitrogen cooled mercury−cadmium−telluride (MCT) detector. In the view mode the total visual magnifications is 360×, which facilitated the locating of a single bead. Data collection is feasible in both the transmission and reflectance modes. In this study the transmission mode was employed.

A well-polished NaCl window was used to collect the background spectrum. A few resin beads were then put on the NaCl window. Using the view mode and the x−y platform of the microscope, the incident radiation was focused on a single resin

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bead. The diameter of the individual bead was measured under the microscope. Data were collected with 4 wavenumber resolution. Sixty-four scans were averaged.

Estimation of the Loading on a Single Bead. A 0.014 mg sample of Merrifield resin beads (loading 1.0 mmol/g) was accurately weighed on a CAHN 26 automatic electrobalance. This sample contained 90 beads with an average size of 50 μm as counted and measured under the microscope. It was calculated that each bead had an average loading of 100 pmol. We repeated this experiment, and this result was reproducible. Similar experiments were carried out for compound **4** (1.05 mmol/g) and compound **1** (1.34 mmol/g). The average bead size for **4** was 88 μm, and the loading on a single bead was 480 pmol. For **1**, the average bead size was 100 μm and the loading on a single bead was 800 pmol.

Determination of the Distribution of Reaction Product on Beads. Single-bead FTIR spectra were recorded for 30 randomly chosen beads from compound **2** and compound **5** and reaction products on Rink Amide resin and NovaSyn TGA. The intensity ratios (absorbance of the product/absorbance of the starting material) for each set of beads were 0.432 ± 0.018, 0.984 ± 0.005, 3.577 ± 0.185, and 1.160 ± 0.009, respectively.

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Supporting Information Available: Determination of the distribution of reaction product on beads and original IR spectra of single beads for generating Figure 2 are available (5 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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