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Monitoring the Progress and the Yield of Solid-Phase Organic Reactions Directly on Resin Supports

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I. Introduction

Combinatorial chemistry[1−12,71] is so far one of the best approaches to accelerating the drug discovery process. Solid-phase synthesis[13,14] has been the major tool to assemble diverse compound libraries, although the liquid-phase synthesis approach has also been used.[15−17] In the pharmaceutical arena, solid-phase organic synthesis (SPOS)[18−24] is receiving extraordinary attention because this methodology provides an approach to synthesizing drug-like small organic molecules.

SPOS is commonly carried out on beaded polymer resins or on grafted polyethylene pins. Organic reactions on solid supports often require conditions quite different from those used in solution. Before a compound library can be made, a time-consuming solid-phase reaction optimization (chemistry validation) process is always required. The optimization process could take months to complete, while the final synthesis of the library would take only weeks. In the optimization process, the selection of linker, building blocks, temperature, reaction time, mixing method, and catalyst all need to be evaluated quickly. Various resins have different microenvironments in terms of hydrophobicity, steric hindrance, and site flexibility. Resin swelling is so crucial for a reaction to occur and reach completion that the selection of the resin and the solvent for a reaction is critical. A fast, simple, and sensitive method (like TLC) would be a very useful tool for rapid feedback in reaction optimization efforts.

A key advantage of SPOS is that it circumvents tedious intermediate isolation and purification procedures such as recrystallization, distillation, and chromatography. Ideally, a solid-phase reaction can be driven to completion by excess reagent without causing problems in the purification. In reality, most organic reactions cannot easily go to completion. Unlike solution synthesis, the resin-bound synthetic intermediate in SPOS cannot be purified. The unreacted portion will accumulate until the end of the synthetic sequence and cause purity problems for the final product. Therefore, on-resin quantitative analysis of solid-supported compounds at intermediate stages is very advantageous in guiding the multistep synthesis.

When no on-resin analytical method is available, the only choice left is to "cleave and analyze". In this practice, the products or intermediates are chemically or photochemically cleaved from the resin and then purified for analysis with classic spectroscopic tools. If compounds are in solution, all conventional analytical tools (MS, NMR, HPLC, TLC, and IR) will be available and the only issue is the capacity. However, to cleave and analyze is a time-consuming, expensive, and laborious process. Some synthetic intermediates are not stable enough for the "cleave and analyze" protocol. It is especially a waste of time and sample for characterizing intermediates in multistep synthesis. Photochemical linkers have simplified this procedure, but the types of reactions that can be carried out on such labile linkers may be limited. The cleavage of diverse compounds from resin supports relies on the chemical linker used during the synthesis. Furthermore, the isolated yield is usually different from the on-resin yield that is important to know for multistep synthesis. It would be more useful to be able to analyze the intermediates or products while still bound to the resin.

The development of analytical methods in support of combinatorial chemistry and SPOS has been an active research area. Recent progress in analytical methods has been summarized in several recent reviews.[25−28] In this account, I summarize progresses in the applications of an on-resin TLC equivalent analytical method—single bead FTIR microspectroscopy (single bead IR)—and on-resin quantitation methods in the monitoring of SPOS. These methods have provided analytical solutions particularly to the chemistry validation process in combinatorial chemistry.

II. Qualitative Analysis of Resin-Bound Compounds

A. Techniques. One of the most useful tools for analyzing the reaction progress in solution is TLC. Even though it only provides qualitative answers, it assists competently in most aspects of reaction optimization and in-process monitoring. The loss of the TLC method in SPOS has caused tremendous problems in the quality control of the reaction. Amine color tests[29−31] that are borrowed from solid-phase peptide synthesis (SPPS) are frequently used

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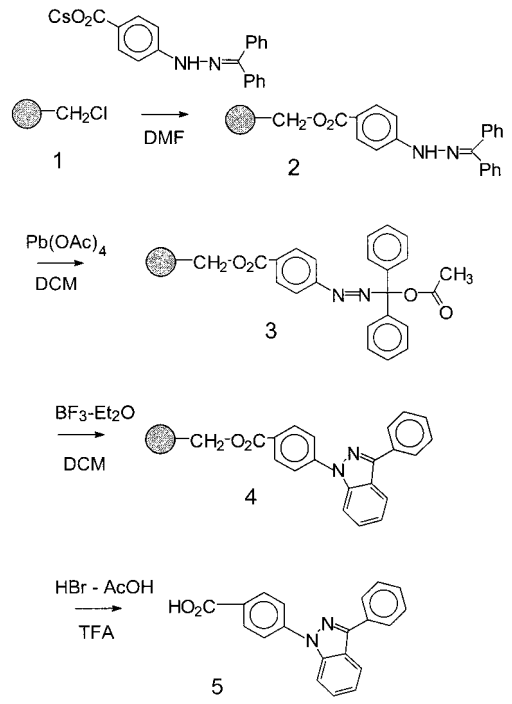
Scheme 1



as qualitative tests when amines are involved in the SPOS. In these kinds of tests, the reaction between the resin-bound amines and a reagent produces a colored product on resin or in solution. On the basis of the color changes, the presence of an amine can be confirmed. Rapid qualitative analysis has been more frequently obtained on ~10 mg resin beads using various techniques such as the KBr pellet method,[32−34] FT Raman,[35] diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS),[36] photoacoustic FTIR spectroscopy,[37] and, alternatively, FTIR microspectroscopy on a single resin bead.[38,39] The principle of monitoring reactions by IR is based on the detection of the functional group interconversions via chemical reaction and appearance or disappearance of functional groups carried by building blocks or protecting groups introduced or removed during the reaction. The functional group to be monitored need not be directly involved in the reaction that is monitored. Therefore, for the rehearsal of a library synthesis on solid supports, building blocks to be used in solid-phase reaction can be selected to contain an IR detectable group at a remote site. The single bead IR method is by far the most appropriate technique for a TLC analogue because of the following: (1) Although a single bead is all we need to record an IR spectrum nondestructively, tens of beads are usually needed for easy handling without resorting delicate single bead handling instruments. The removal and examination of only tens of beads from the reaction suspension practically does not perturb the reaction so that the reaction is monitored in real time; (2) a high-quality spectrum can be recorded within a minute; (3) no sample preparation is required, reducing the whole analysis time from sampling to results to a few minutes (even faster than TLC); (4) a computerized automated microscope stage for high-throughput analysis is now commercially available,

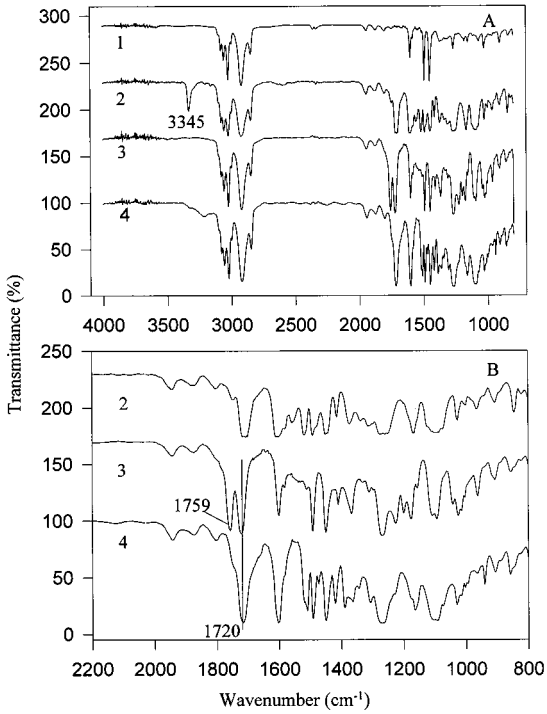


FIGURE 1. Single bead IR spectra were taken as previously described. This reaction series starts with Merrifield resin. The spectra labeled 1−4 are for compounds **1**−**4**, respectively. Panel B shows spectra of **2**−**4** on an expanded scale from 800 to 2200 cm^{-1}.

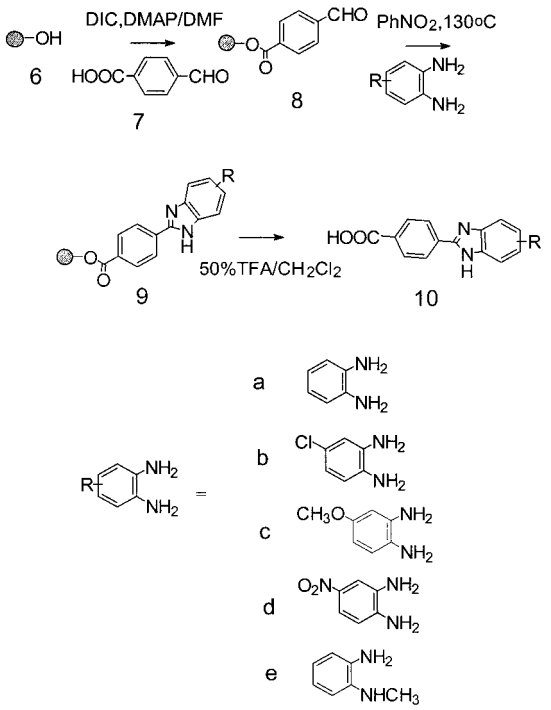
enhancing the application of this technique. The cost of the IR microscope is probably the only concern for average organic chemistry laboratories. A study searching for a low-cost substitute of this technique is in progress in my laboratory.

B. Examples. An indazole synthesis (Scheme 1) was monitored by single bead IR.[40] Shown in Figure 1, the coupling of benzophenone−(4-carboxyphenyl)hydrazone to the Merrifield resin **1** was unambiguously identified by the appearance of many spectral features in **2**. The sharp peak at 3345 cm^{-1} is assignable to the N−H stretch of the hydrazone. A very strong carbonyl band also appears at 1710 cm^{-1} corresponding to the aromatic ester functionality introduced in this step. In addition, prominent C−O−C skeletal vibrations emerged at 1280 and 1100 cm^{-1}. The quantitative oxidization of the hydrazone **2** to the (α-arylazo−benzhydryl acetate **3** by $Pb(OAc)_4$ is indicated by the complete disappearance of the N−H band, due to oxidation of the hydrazone to the azo-functionality and the appearance of a second well-resolved carbonyl stretch at 1759 cm^{-1}. The latter is assignable to the stretch of the carbonyl in the newly formed aliphatic ester. The cleavage of the N,O-acetal **3** by BF_3 and the successful formation of resin-bound indazole **4** is supported by the disappearance of this carbonyl band.

III. Semiquantitative and Quantitative Analysis of Resin-Bound Compounds

A. Nonspectroscopic Methods. Since the resin-bound intermediate cannot be purified, the completion of every reaction step must be confirmed by an effective analytical

Scheme 2



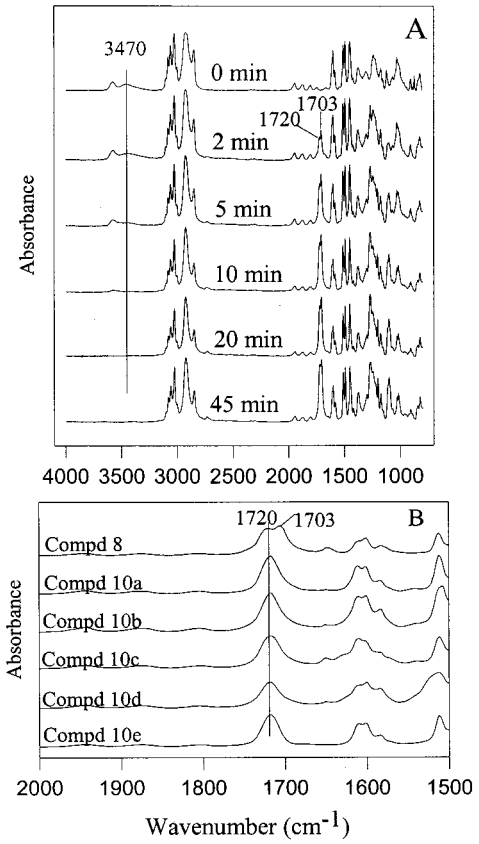


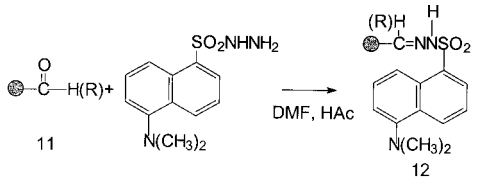
FIGURE 2. (A) Single bead IR spectra at various times during the synthesis of **8**. The starting resin is Wang resin. (B) Single bead IR spectra for resin-bound product **10a**−**e**. Five sealed tubes with dried resin (100 mg, 1.0 mmol/g), one of five amines a−e (1 mmol), and 3 mL of nitrobenzene were heated at 130 °C overnight. After they were cooled to room temperature, the resins were transferred to the filtration tubes and washed. Single bead IR spectra were taken for each product.

method. Methods for quantifying amine groups have been reported in SPPS.[29−31] The conventional combustion elemental analysis, acid−base titration, and FTIR can also provide semiquantitative information. Polymer resin beads can be burned completely and the percentage of one or more elements determined by various analytical methods fairly accurately. The reaction yield or completion can be assessed on the basis of the results. To evaluate the general utility of this method, we have carried out a multiple analysis of 16 resin samples for their carbon, hydrogen, nitrogen, sulfur, chlorine, bromine, and iodine contents. Results showed that the reproducibility of the method is remarkable (<3% variations) and the relative error from the expected value is <5% for most of the samples.[72] This method has aided the quantitative estimate of the reaction yield. However, it is a destructive method and consumes relatively large amounts of resin (at least ∼5−50 mg). Other potential problems for this method are as follows: (1) the element to be analyzed is significantly "diluted" by resin matrix, and (2) the sample sometimes cannot be completely washed free from entraining interfering reagent and solvents, which interfere with the quantitation.

Gravimetric analysis extracts reaction yield information from the weight gain of the resin after a reaction. Results obtained from the gravimetric analysis for quantitation is in general misleading. This is because (1) the weight gain in each step of synthesis is too small to detect relative to the dominating resin weight and (2) many solvents, reagents, and even unknown impurities are easily trapped into the resin bead, causing nonpredictable weight variations. Acid/base titration has been used to quantitate the loading of resin samples.[41,42] In the polymer matrix, the accumulation of negative charges can inhibit the further complete titration of the remaining acid groups with a base. For this reason, the accuracy of this method is questionable and needs further investigation.

B. Single Bead IR Method. In TLC analysis, the disappearance of the starting material spot accompanying the formation of the product spot tells us when the reaction is complete. If the disappearance of an IR band from the starting material accompanies the formation of product bands, the percentage decrease in the IR band can be used to estimate the conversion in this reaction step. For example, the conversion from **2** to **3** (Scheme 1) is nearly quantitative as seen from the total disappearance of the band at 3345 cm^{-1} in this conversion (Figure 1). The percentage conversion of a solid-phase benzimidazole synthesis (Scheme 2) was also monitored by the single bead IR method.[43] The IR spectra taken at various times during the first step in Scheme 2 are shown in Figure 2A. Among other spectral features, two distinct IR bands are introduced: an ester carbonyl band at 1720 cm^{-1} and an aldehyde carbonyl band at 1703 cm^{-1}. At the same time, the hydroxyl band at ∼3500 is diminishing. In about 20 min, the intensity of the hydroxyl band almost reaches zero and those of the carbonyl bands reach a constant maximum value suggesting a quantitative conversion. The

Scheme 3



yield of 97% for this transformation was independently determined directly on the resin using a 6-mg resin sample and a novel fluorescence method.[44] A series of benzimidazoles **10a**−**e** were then synthesized. The reaction was carried out at 130 °C for 8 h using nitrobenzene as solvent. The success of this reaction is confirmed by the disappearance of the aldehyde carbonyl band at 1703 cm^{-1} (Figure 2B) and the formation of the N−H stretch for **10a**−**d** at ∼3400 cm^{-1}. Due to the overlap between the ester and aldehyde carbonyl bands (Figure 2B), the ratio of the two bands (1703/1720) in compounds **10a**−**e** was determined by peak-fit analysis to obtain the on-resin purity and the yield of the product.[43] This method has been successfully applied to quantify reactions in which the starting resin material has an IR detectable functional group that is involved directly or indirectly in the reaction. In another approach,[45] if a deuterium isotope-labeled protecting group is used in the synthesis; then the protection/deprotection step can be quantitated on the basis of a separately determined calibration curve. This method would require special isotope synthesis.

C. UV and Fluorescence Methods. A known amount of fluorescent dye specific to an organic functional group reacts with resin bound compounds, and the depletion of the dye in the supernatant is then determined by UV−vis or fluorescence spectroscopy as an indication of the absolute amount of compounds on-resin. In principle, this strategy can be applied to many organic functional groups. It was first used to determine the absolute amount of aldehyde and nonhindered ketone groups[44] directly on resin supports (Scheme 3). Approximately a 2-fold excess of dansylhydrazine was mixed with DMF-swollen aldehyde/ketone and the plain polystyrene (as a control) resins for 30−50 min. The amount of aldehyde/ketone groups was calculated from the reduced fluorescence peak areas or UV absorbance. The portion of noncovalently resin-trapped dye molecules are corrected for by subtracting the amount of dye molecules consumed on the identical amount of plain polystyrene resins. The completion of the coupling reaction was confirmed by single bead IR in all cases. Figure 3 shows supernatant fluorescence spectra and the single bead IR spectra taken at various times during the coupling reaction (Scheme 3). This principle has also been applied to develop quantitation methods for other organic functional groups such as hydroxyl and carboxyl resins.[73] This method works equally well for polystyrene (PS) and poly(ethylene glycol)−polystyrene (PEG−PS) resins. The technique, however, cannot ensure a reaction is 97% complete or 99% as such quantitations are often required for repetitive synthesis such as peptoid synthesis.

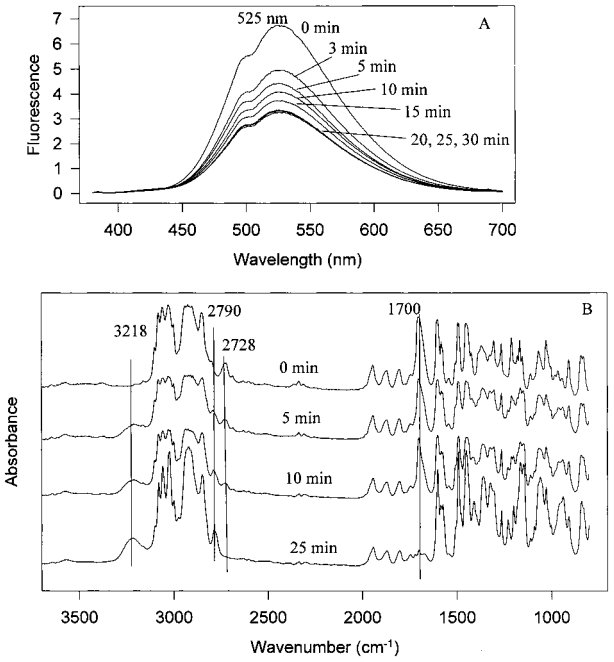


FIGURE 3. (A) Fluorescence emission spectrum of hydrazine in the supernatant and (B) single bead IR spectra taken out of the reaction mixture at various times during the reaction of formylpolystyrene resin with dansylhydrazine.

IV. Reaction Kinetics on Resin Supports

To fully realize the advantage of SPOS in multistep organic synthesis, the kinetics of solid-phase reactions needs to be known. Reactions should go to completion, preferably at a high rate, particularly at intermediate steps to ensure a relatively high purity in the final product. However, there is a serious lack of kinetic studies for polymer-supported reactions. There are two reasons for this. First, the general lack of analytical tools for on-resin analysis in the past has limited our capability to pursue such studies. Second, some available methods (such as gel-phase NMR or KBr pellet FTIR) would require too much sample (5−10 mg) for a single time point. This is impractical for general use in kinetic studies considering the small-scale synthesis and the cost of resins. Even though no data exist to support it, reactions on polymer supports are frequently assumed to suffer from steric hindrance and, therefore, to be slower compared with their solution counterparts. As a consequence, solid-phase organic reactions are commonly carried out for 12−24 h indiscriminately before cleaving compounds off for analysis.

A. Techniques for Kinetic Studies. In peptide synthesis, the task is simpler since only amino acid coupling and deprotection reactions are involved. The change in the concentration of the protecting group such as Fmoc with time can be monitored as the reaction time course for a deprotection reaction. This was incorporated into a synthesizer for the on-line monitoring of the synthesis of peptides.[46] A method based on the incorporation of dansyl groups into free amine and followed by hydrazinolysis of the ester bond can quantify the change in free amine groups in amino acid coupling reactions.[47] In

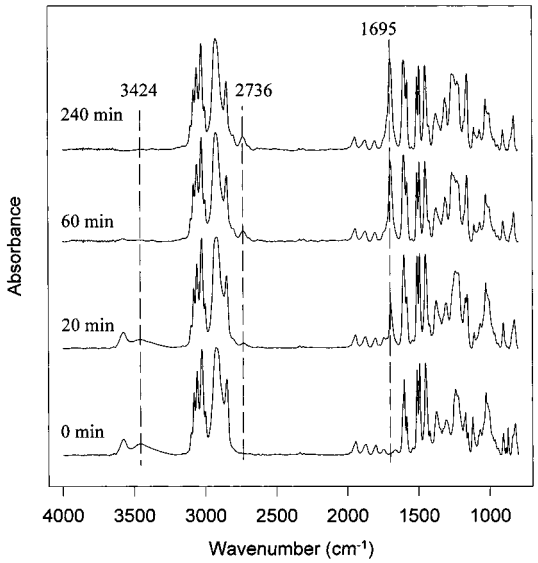
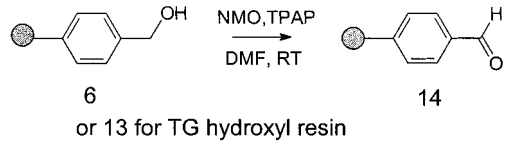


FIGURE 4. IR spectra taken from a single bead at various times in the course of Scheme 4. Spectra were taken from a single flattened bead at 0, 20, 60, and 240 min after the initiation of the oxidation reaction. The hydrogen-bonded and unbonded hydroxyl stretch near 3400−3600 cm^{-1} disappears as the intensities of the bands for the aldehyde C−H (2736 cm^{-1}) and the aldehyde carbonyl (1695 cm^{-1}) increase.

Scheme 4



6 14

or 13 for TG hydroxyl resin

another method, the reaction of 2-hydroxy-1-naphthaldehyde and resin-bound free amine groups to form a Schiff's base using 10−50 mg of resin and 12 h reaction time in a two-step reaction was also used to quantify the change of the amount of amine groups on the resin. According to this study, the coupling of some protected amino acids (i.e., Boc-isoleucine) was extremely slow.[48] An acid−base indicator bromophenol blue was used to quantitate free amine groups.[42] The free amine quantitation methods described in the previous section can be used to quantify the time course of peptide synthesis. Volhard titration of chlorine was used to quantify the time course of synthetic reactions starting with Merrifield resin with ~200 mg resin per time point.[49] The single bead IR method is capable of providing kinetic information on solid-phase organic reactions in real time without perturbing the reaction. Reaction kinetics of many reactions have been studied using single bead IR.[50,51] Difference spectra between the product and the starting material or between the resin-bound compound and polystyrene resins are easily obtained. However, difference spectroscopy was rarely needed in reality. This is because the polystyrene bands serve as convenient internal standards for quantitation. In quantitative analysis, normalization of a polystyrene band at 1945 cm^{-1} for spectra taken from various beads is necessary to correct for the bead size and path length variations.

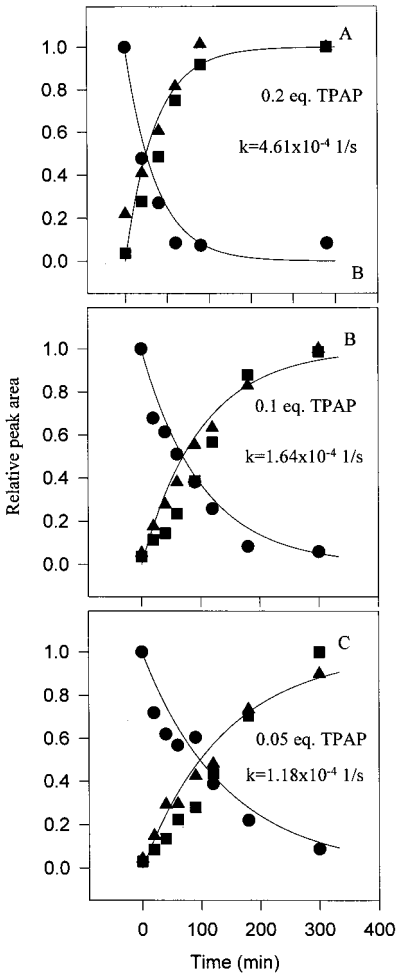


FIGURE 5. Time course of the reaction in Scheme 4. All spectra were normalized by making the intensity of the polystyrene band at 1945 cm^{-1} equal. The area integration for the hydroxyl band from 3181 to 3637 cm^{-1} (circles), the aldehyde C−H band from 2664 to 2766 cm^{-1} (squares), and the aldehyde carbonyl band from 1641 to 1765 cm^{-1} (triangles) for spectra at various times were plotted against time. Lines were calculated from the best fit to a first-order reaction equation with a rate constant shown. The amount of catalyst TPAP was 0.2 equiv in A, 0.1 equiv in B, and 0.05 equiv in C.

B. Examples. In Scheme 4, the catalytic oxidation of **6** to a resin-bound aldehyde **14** was monitored by single bead IR (Figure 4). The areas of IR bands which undergo changes were integrated and plotted against time (Figure 5). The data are fit by a pseudo-first-order rate equation to obtain rate constants as shown in Figure 5 and the rate depends on the amount of catalyst tetra-*n*-propylammonium perruthenate (TPAP) used for the oxidation (Figure 5).

Attenuated total reflection (ATR) or internal reflection is a versatile, nondestructive technique for obtaining IR spectra from the sample surface. When the light is totally reflected at the interface between two media of different refractive indices, an evanescent wave in the less dense medium extends beyond the reflecting interface. IR spectra of the less dense medium can thus be conveniently obtained. Using an ATR objective in the same IR microscope for recording the single bead transmission IR spectra, an IR spectrum from the partial surface of a single

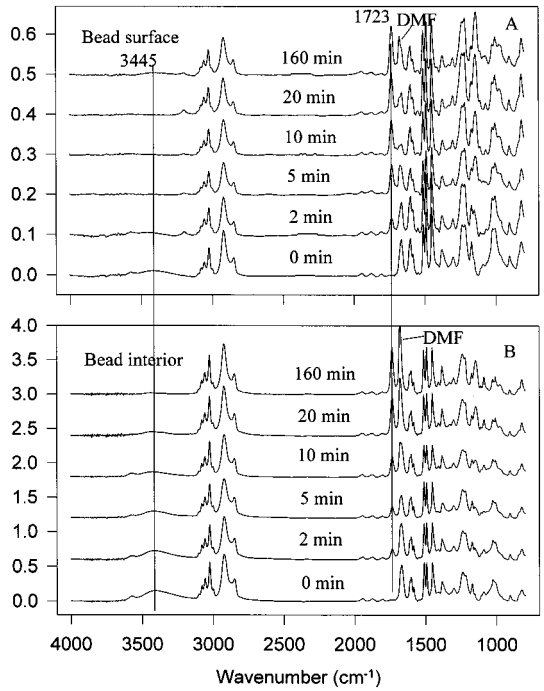
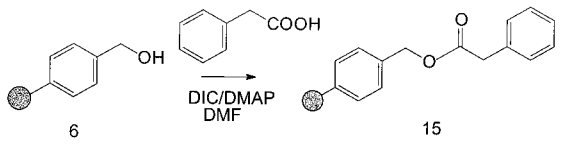
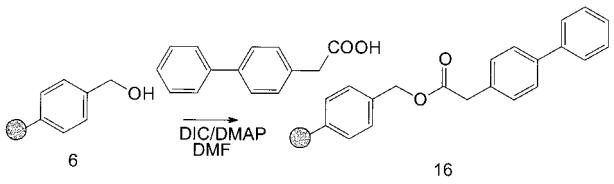


FIGURE 6. IR spectra from a single bead taken at specified times during the course of the reaction in Scheme 5. Spectra taken from a partial surface of a single bead are shown in A and from the whole bead (99% is bead interior) in B. The amount of compound sampled is calculated to be 130 fmol for the surface measurement and ~500 pmol for the whole bead experiments. IR absorbance bands attributable to the disappearing hydroxyl group at 3445 cm^{-1} and the emerging carbonyl group at 1738 cm^{-1} are highlighted with the lines.

Scheme 5

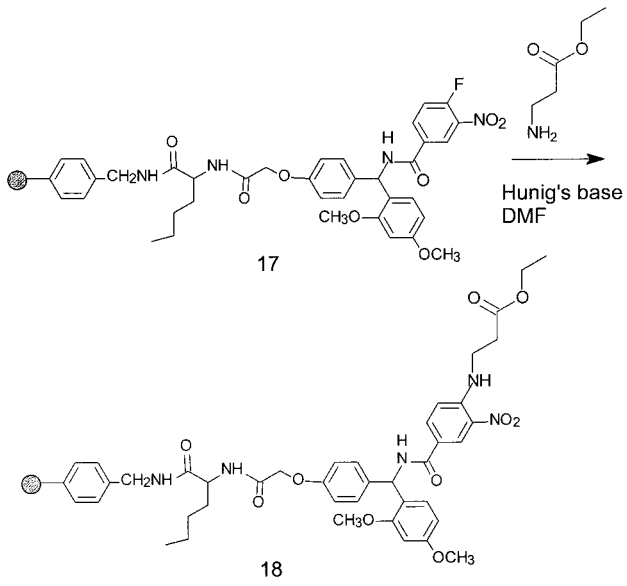


Scheme 6



bead can be obtained within a couple of minutes. To investigate the problem of the possibly hindered diffusion of a reagent into the bead interior, the reaction rate on the bead surface and in the bead interior was monitored simultaneously using ATR mode and transmission mode, respectively. Spectra taken from a single bead surface and interior at various times during the reaction in Scheme 5 are shown in Figure 6. Reaction time courses on the bead surface and in the bead interior for several reactions (Schemes 5−7) were also studied,[50] and no difference was found in the reaction rate on the surface and in the interior of the bead. Reaction on a single bead can also be monitored in situ using a flow-through cell.[52,53] In this approach, the spectra have to be recorded in the presence of solvent. Since the IR absorption band of a solvent

Scheme 7



interferes with reaction monitoring, most solvents used in SPOS cannot be used in this method. With an IR-friendly solvent (in most cases, CH_2Cl_2), real-time monitoring can be achieved.

V. Effects of Resin and Reaction Conditions on Solid-Supported Organic Reactions

A. Effect of Resin Supports. The selection of the optimal resin for synthesis is important when planning a library synthesis. The two most commonly used resin supports are PS- and (PEG−PS-based resins. In all solid-phase synthesis papers, they are both indistinguishably shown as a black ball. Actually their chemical structures are quite different (Figure 7). PS contains mainly 1% divinylbenzene (DVB) cross-linked polystyrene backbones (~96−98% in weight) which is highly hydrophobic. The short linkers and reacting groups constitute only ~2−4% of resin weight. Due to the short linker length, reactions on PS resin tend to be affected more by the hydrophobic PS backbones. Although most peptide synthesis has been

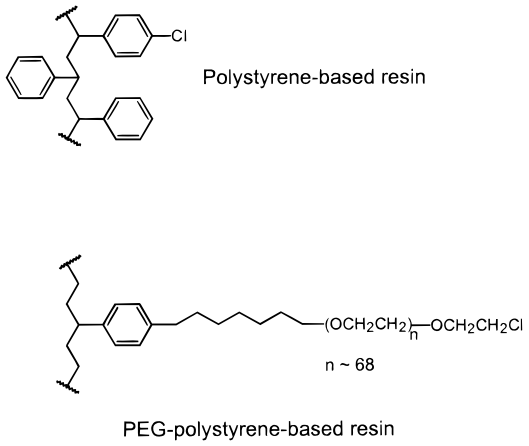


FIGURE 7. Comparison of the chemical structures of PS- and PEG−PS-based resins.

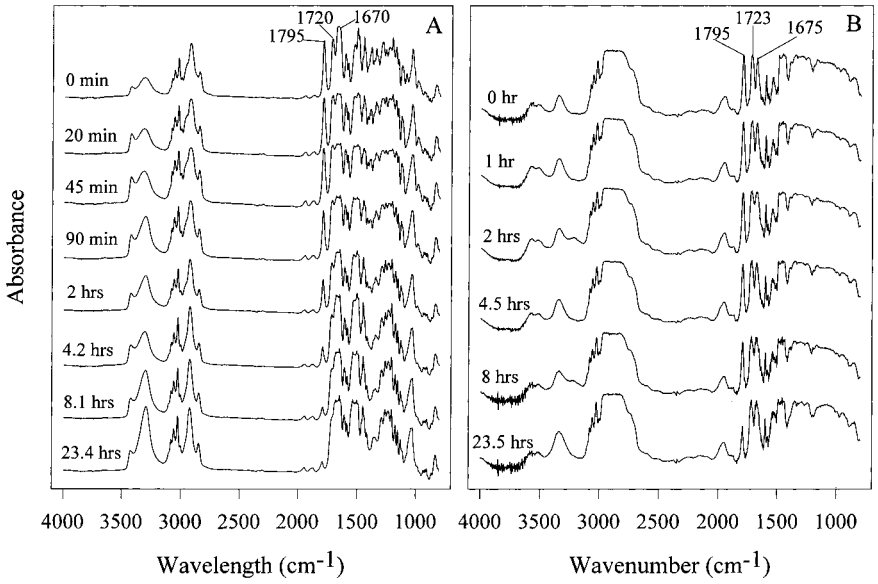
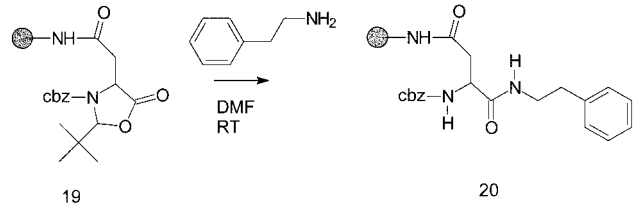
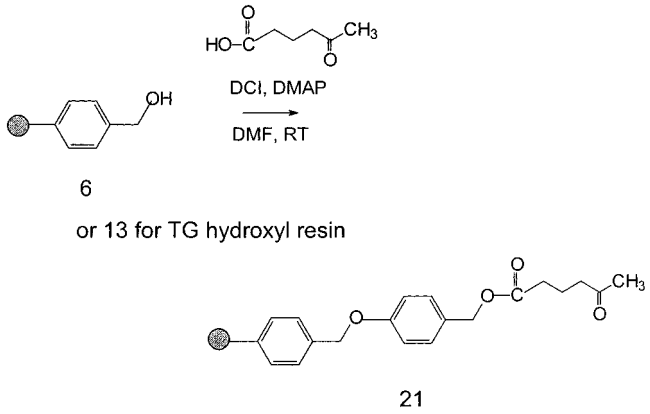


FIGURE 8. IR spectra taken from a single flattened PS bead (A) or a single PEG−PS bead (B) at various times during the reaction in Scheme 8.

Scheme 8



Scheme 9



performed on PS resins, this resin may not be the optimal support since protected amino acid reactants are relatively polar.

The architecture of PEG−PS resin is based on a very small portion of cross-linked 1% DVB−PS backbones extensively grafted with long PEG linkers (>60 ethylene oxide units). The PEG content is up to 70−80% of the resin weight. Therefore, the mechanical, physicochemical behavior of the resin is determined by the PEG chain. Because the reactive sites in PEG−PS resins are located at the end of long and flexible spacers and totally separated from the PS backbone, they are less affected by PS matrix. Additionally, the dominant PEG linkers exhibit greater miscibility with most solvents including water. These are why PEG−PS is generally regarded as providing a quasi-homogeneous and "solution-like" reaction support. It is commonly assumed that reactions carried out on PEG−PS resins proceed faster than those carried out on PS resins. Peptide synthesis reactions would be more favored on PEG−PS resins since it is more polar than PS resins. However, peptide bond formation is only a special reaction. In SPOS, a different set of rules may apply.

To compare the reaction rate on PS and PEG−PS resins, several reactions were carried out on these two resins under identical conditions. Figure 8 shows two sets of single bead IR spectra taken at various times during the reaction shown in Scheme 8. A related solid-phase synthesis via 5-oxazolidinones was described earlier.[54] In this kinetic study,[55] the ring-opening reaction on both PS and PEG−PS resin was monitored by following the diminishing rate of the IR band at 1795 cm^{-1}. The reaction on PS resin is 18 times faster than that on PEG−PS resin. These data and results from studies on other reactions (Schemes 3, 4, and 9) are listed in Table 1.[55] Recently Czarnik[56] has summarized some key observations including ours on this subject and pointed out that solid supports are like solvents. The effect of polymer matrix on the reaction rate is similar to the effect of solvent on a solution reaction rate. In our working hypothesis, the reaction rate on resin supports should be proportional to the compatibility of the reagent with the solvent and the adsorption coefficient of the reagent into the resin. The selective adsorption of a compound into the resin depends on its similarity to the solvated resin in terms of

Table 1. Comparison of Reaction Rates on PS- and TG-Based Resins

reacn scheme	k_{PS} (1/s)	k_{TG} (1/s)	k_{TG}/k_{PS}
4	$4.6 \times 10^{-4*}$	1.8×10^{-3}	3.9
9	2.2×10^{-4}	2.3×10^{-4}	1.0
3 (R = H)	3.1×10^{-3}	1.8×10^{-3}	0.6
3 (compd **21**)	4.1×10^{-4}	1.9×10^{-4}	0.5
8	1.13×10^{-4}	6.26×10^{-6}	0.055

molecular interactions such as hydrogen bonding, hydrophobicity, and polarity. The enhanced reaction rate and efficiency when phase-transfer catalysts were used[57−59] provide support for this hypothesis. To summarize this section, there is no single polymer support that favors all reactions. Depending on whether the SPOS requires polar or nonpolar medium, PEG−PS- or PS-based resins, respectively, should be chosen.

B. Effect of Mixing Methods. SPOS offers several advantages over solution-phase techniques for achieving high-throughput synthesis. At the same time, SPOS also poses problems that do not exist in solution synthesis. One of the problems is that one has to select the most efficient reaction mixing method to maximize encounters between the solid-bound reactant and the soluble reagent. Diverse mixing techniques are currently used in various laboratories without a clear understanding of their relative efficiency. Bead suspensions are usually mixed by shaking the tubes, which is fixed at a 45° angle, horizontally by an orbit shaker, by angular shaking (20−30°) on a wrist shaker, by continuously rotating tubes at an angle of 180° or 360° (whole turn) rotations at a certain rpm, by bubbling N_2 gas from the bottom of the reactor, and by the conventional magnetic stirring. To compare the mixing efficiency, the reaction in Scheme 3 was studied using six different mixing methods.[60] Single bead IR and the fluorescence quantitation method[44] were used to monitor the reaction completeness. For the reaction studied, the 360° rotation and nitrogen bubbling methods provided high mixing efficiency in solid-phase synthesis under mild conditions. Wrist shaking also showed high mixing efficiency with the maximum setting (vigorous shaking). Magnetic stirring showed the same high mixing efficiency, but this method often breaks beads. The method with 180° rotation mixing required longer reaction time and may not be preferable for slow reactions. Mixing with an orbit shaker did not give satisfactory reaction yields as compared to those obtained by the other techniques unless a large excess of reagent and a prolonged reaction time are used. In the same way, the optimal solvent, building block, linker, catalyst, and other conditions have been routinely selected quickly with the aid of single bead IR screening.

C. Effect of Site−Site Interactions. An important advantage of solid-phase organic synthesis is that, in principle, compounds can be isolated from each other when attached to the solid support. This "pseudo-dilution" effect should be very useful for carrying out reactions that are impossible in solution, e.g. intramolecular cyclizations without the problems caused by intermolecular reactions and mono-derivatization of bifunctional molecules. While this pseudo-dilution effect has been found to be true in some cases,[61−63] the rule was broken in others.[64−69] For a specific reaction, the proper resin can be selected in order to promote or reduce the site−site interactions that generally exist in resin supports. Site−site interactions cannot be totally eliminated even at higher cross-linking and lower loading, although they are affected very much by factors such as

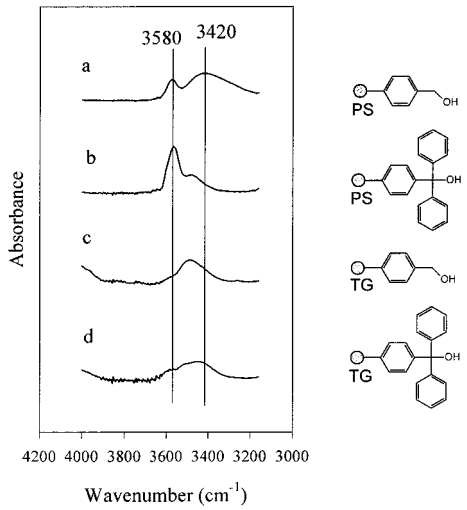


FIGURE 9. IR spectra taken from a single flattened bead for resins shown on the right; (a) Wang resin; (b) tritylhydroxyl resin; (c) PEG−PS hydroxyl resin; (d) PEG−PS tritylhydroxyl resin. In PS resins, the intensity of the free hydroxyl band at 3580 cm^{-1} increases as the microenvironment of the hydroxyl group becomes more bulky. In PEG−PS resins, since site interactions are extensive, almost no free hydroxyl groups exist.

reactivity of reagents used, available sites, and the existence of steric hindrance.[70] Figure 9 shows the site-interaction/site-separation in different hydroxyl resins or on different linkers in the same hydroxyl resin. In these examples, the hydroxyl band is divided into the free (a sharp band at 3580 cm^{-1}) and the hydrogen-bonded (broad band ∼3420 cm^{-1}) hydroxyl group fractions. PEG−PS resin usually affords no site separation at all and shows only the hydrogen-bonded hydroxyl band (Figure 9c,d). PS resin always allows some site separation, and both bands are observed (Figure 9a). Sterically hindered linkers increase the chance for site isolation (Figure 9b).[70]

VI. Concluding Remarks

Combinatorial library synthesis requires the development of reliable and robust solid-phase organic reactions. For a majority of reactions to be monitored in reaction optimization stage, the main task is to confirm the intended structure rather than a full structural elucidation of an unknown. For this reason, a confirmation of the desired functional group changes by FTIR are usually sufficient in most cases. Single bead IR has been used as a TLC-equivalent analytical method because it detects a broad range of spectral changes and provides a sensitive, rapid, convenient, and real-time analysis. This technique provides qualitative, quantitative, and time-resolved information on solid-phase organic reactions.

It is imperative to be able to quantify all resin-bound intermediates directly on the resin. When an IR band of the starting material disappears accompanying the formation of the product, the percentage conversion of the reaction can be estimated quickly on the basis of peak area changes. The dye-coupling-and-consumption method is a rapid quantitation method for obtaining the absolute amount of functional groups on the resin. When no

spectroscopic methods can be applied and a reaction involves changes in compounds containing nitrogen, sulfur, or halides, combustion elemental analysis is a useful method in many instances. However, quantitative analytical methods for monitoring solid-phase organic reactions are still severely lacking. Development of more rapid, on-resin quantitation methods for analyzing diverse organic functional groups will be one of the major challenges for years to come.

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