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Direct Monitoring of Organic Reactions on Polymeric Supports

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Abstract: A method to use matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) for real-time monitoring of organic reactions on polymeric supports used in solid-phase synthesis is described. The strategy utilizes a synthetic construct that allows for the rapid and convenient direct MALDI analysis of the attached substrates as well as their subsequent chemical cleavage as desired. We have used this strategy to monitor nucleophilic substitutions, palladium-catalyzed coupling reactions, and solid phase peptide synthesis reactions using both Boc- and Fmoc-based chemistries.
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Organic synthesis on polymeric supports, commonly known as solid phase synthesis,[2a] offers several advantages over solution-based techniques, including the ability to use concentrated reagents in large excess, to easily isolate products, and to utilize solvents in which the reactants alone would be insoluble.[3] Solid phase techniques have been exploited for many years in the synthesis of peptides[2] and oligonucleotides[4] and more recently have proven invaluable in oligosaccharide[5] synthesis as well as in many areas of combinatorial chemistry.[6] However, one serious drawback of the use of insoluble supports in organic synthesis is the difficulty associated with monitoring the progress of reactions and characterizing resin-bound reaction products. Although NMR[7] and IR[8] spectroscopy have been used with some success to characterize resin-bound molecules, no convenient and general techniques exist for the routine monitoring of organic reactions on the solid phase.

Recently mass spectrometry (MS) has also proven useful for the analysis of resin-bound molecules.[9] However, in most cases the application of MS to real-time solid phase reaction monitoring has been limited by the need for cleavage of the molecules from the resin[9b-g] or the inefficient ionization of the analytes.[9a,h] More recently, Fitzgerald *et al.*[10] described a method where resin beads derivatized with a photocleavable linker and then carried through cycles of solid phase peptide synthesis (SPPS) could be analyzed directly using MALDI-MS. Although this approach did not require cleavage of the peptides from the resin prior to MALDI analysis, it failed in the cases of fully-protected peptides, which apparently lacked sufficient protonation sites to effect their ionization during MALDI analysis. This same limitation would also apply to many small molecule organic compounds.

We have developed a simple, general method for the real-time analysis of small molecule organic reactions on solid supports. Our method relies on the use of a derivatized resin that incorporates a dual linker strategy[11] and an ionization tag[12] (Figure 1). A photocleavable linker is used to attach an ionization tag sequence to the resin support. A chemically cleavable linker is then added to provide for synthesis and release of the target molecules. The combination of the photocleavable linker and the ionization tag enables direct analysis of the resin beads by MALDI-MS regardless of whether the attached reactants or products contain potential protonation sites for MALDI. Additionally, the photocleavable linker/ionization tag combination provides a sufficient add-on mass (~530 Da) so that the spectral peaks from small molecule synthetic organic compounds are not obscured by low molecular weight matrix peaks (< 500 Da). At the completion of the synthetic manipulations, the chemically-cleavable linker permits recovery of the desired products while leaving the ionization tag and photocleavable linker sequences on the insoluble resin. The modular nature of this approach also allows it to be tailored for different chemistries by the use of a variety of linkers and ionization tags as well as solid supports. To date we have used a positively-charged ionization tag with various combinations of resins, photocleavable linkers, and chemically-cleavable linkers.

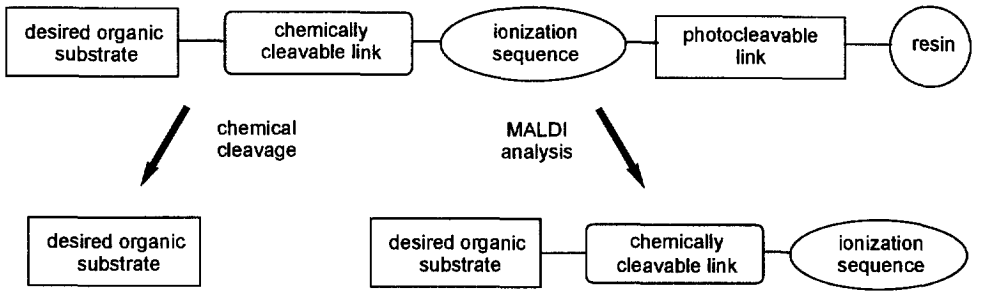
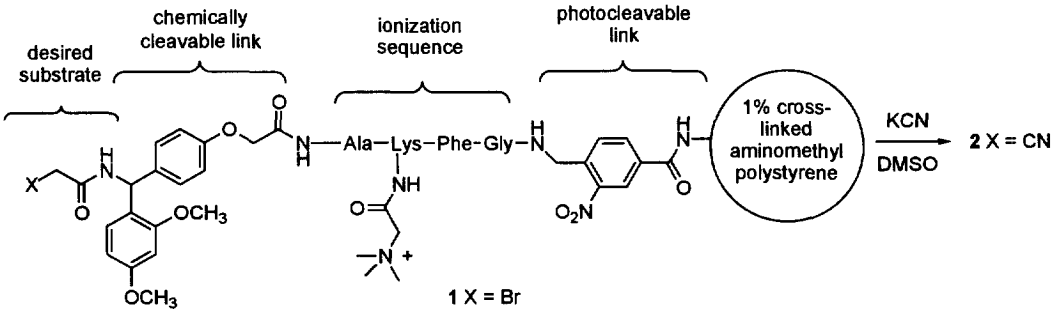


Figure 1. Schematic representation of the resin-bound construct and the resulting products after chemical cleavage and photochemical cleavage (during MALDI analysis).

Here we illustrate how the strategy outlined above was used to monitor the reaction of resin-bound bromoacetamide with potassium cyanide (~ 10 eq) in DMSO (Scheme 1).[13] MALDI analysis of the reaction at 0 and 2 min is shown in Figure 2. At each time point ~50-100 resin beads[16] were removed, washed with DMF (1 x 30 s), washed with 1:1 MeOH/CH_2Cl_2 (1 x 30 s), and briefly dried (~1 min). The beads were then suspended in 5-10 μl of a saturated solution of *trans*-3-indoleacrylic acid (a common MALDI matrix chemical[17]) in 1:1 CH_3CN/H_2O with 0.1% TFA. A 1.3 μl aliquot of this solution was placed on a stainless steel sample plate, air dried, and subjected to MALDI analysis.[18] The total elapsed time for this procedure was < 10 min. The spectra in Figure 2 indicate that complete conversion to the nitrile was effected in 2 min. At t = 0 min the major species detected is peak A (m/z = 941.2), corresponding to the product expected from the photocleavage of **1** (calc. MW for MH^+ = 941.7 Da). At t = 2 min two different signals are detected. The major species, peak B (m/z = 889.6), corresponds to the product expected from the photocleavage of **2** (calc. MW for MH^+ = 887.9 Da). The minor species, peak C (m/z = 904.6), is attributed to a $(MH + 15)^+$ adduct ion of peak B resulting from the desorption process, although the identity of the species is unknown.[19] We verified that the MALDI spectra accurately represented the reaction progress by chemically cleaving (TFA/CH_2Cl_2, 9:1, 20 min) 50 mg of resin from the t = 2 min time point and analyzing the resulting solution by NMR. No 2-bromoacetamide methylene peak (δ = 3.8 ppm, DMSO-d_6) was seen, while the corresponding 2-cyanoacetamide methylene peak (δ = 3.6 ppm, DMSO-d_6) was clearly visible (data not shown). We have used this approach to follow other nucleophilic displacements and a Heck reaction[20] on the solid phase as well as individual amino acid coupling reactions in SPPS using both Boc- and Fmoc-based chemistries. In all cases we have been able to quickly and efficiently generate MALDI spectra from the resin samples.



Scheme 1

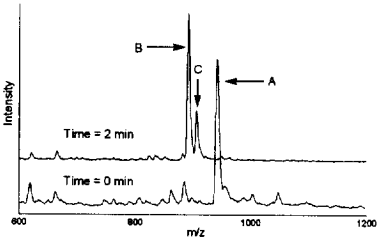


Figure 2. MALDI mass spectra of resin samples removed at time = 0 and 2 min during the conversion of **1** (peak A) to **2** (peak B).

An important feature of our approach is that the desired final products can be isolated free of the linker sequences. For example, peptides synthesized by SPPS using our linker were chemically cleaved from the resin and analyzed by HPLC and ESI-MS to assess their purity. The desired peptides were cleanly separated from the linker/ionization tag sequences, which appear by MALDI analysis to remain firmly attached to the resin. Thus the ionization tag/photocleavable linker combination indeed allows identification of the resin-bound products at any stage of the syntheses without interfering with subsequent isolation of the products released into solution.

The approach we have developed can be used to rapidly and routinely monitor organic reactions on solid supports. Similar to the use of thin layer chromatography for reactions in solution, this technique will allow convenient study of myriad reactions that previously would have required extensive protocols for analysis. The ability to obtain approximate product ratios and mass spectral information in real-time for resin-bound substrates should prove invaluable for solid phase strategies to synthesize small organic compounds, peptides, oligosaccharides, oligonucleotides, and even combinatorial libraries. Work to expand the repertoire of useful linkers, resins, and ionization tags for broad application of the principles described in this work is in progress.

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19. In our spectra we find varying amounts of signal at $(MH+15)^+$ for any given substrate. Separate solution photolyses (354 nm, DMF, 1h) followed by ESI-MS analysis, along with other experiments, have indicated that these peaks are an artifact of the MALDI process. Our experiments have also shown that the extent of adduct formation is highly dependent on a combination of factors including the chemical nature of the substrate and photocleavable link as well as the MALDI matrix used.
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