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Nondestructive, Colorimetric Monitoring of Amines and Thiols on a Solid Support

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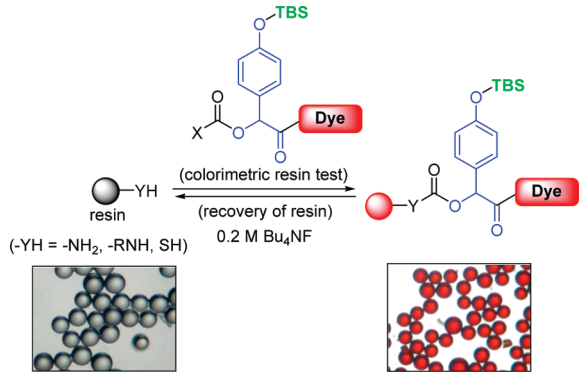
ABSTRACT



A new, nondestructive, highly sensitive method for colorimetric monitoring of primary amines, secondary amines, and thiols on a solid support was developed. The resin used in this method is simply regenerated for the repetition of the reaction or an ensuing reaction. By using this new method, several peptides containing secondary amide linkages and *C*-terminal hydrazide groups were prepared in high purities and yields.

Since the seminal technology of solid-phase peptide synthesis (SPPS) was developed by Merrifield in 1963,[1] solid-phase synthesis (SPS) has become the leading edge technology for the preparation of various substances such as peptides/proteins, unnatural biopolymers, oligonucleotides, carbohydrates, and small molecules.[2] Solid support-based chemistry has several practical advantages over conventional solution-based chemistry, including the fact that (1) SPS does not require implementation of extensive workup procedures and chromatographic purifications and (2) large excesses of reagents can be used to drive reactions to completion. However, the rapid, reliable monitoring of the progress of on-resin reactions remains a significant problem.

Over the past decade, much effort has been devoted to the development of detection methods for solid-phase reactions. One technique involves HPLC analysis of synthetic intermediates that are cleaved from the solid support. Although facile, this method requires relatively large amounts of samples that have to be discarded after analysis. Single-bead FT-IR analysis can also be used to monitor on-resin reactions. However, this method is restricted to analysis of a limited set of substances containing IR discernible functional groups, and it does not enable the reuse of analyzed samples.[3] Nondestructive, NMR spectroscopic detection methods have been developed to analyze the progress of

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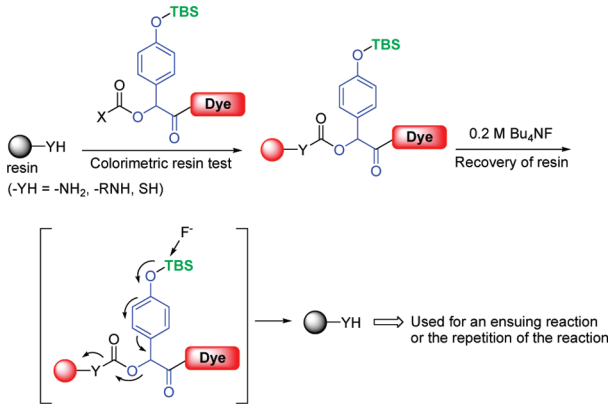


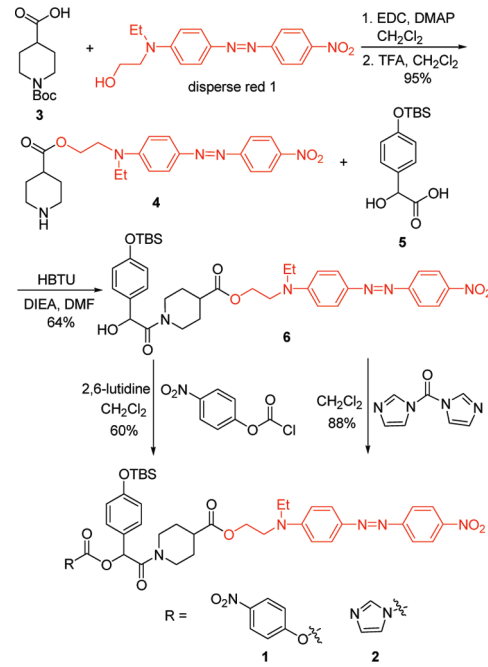
Figure 1. Strategy for a nondestructive colorimetric resin test: resin-bound amines and thiols are monitored by reacting with an activated ester of a reagent, and after the resin test a dye moiety is removed by treatment with Bu_4NF to regenerate the resin via a 1,6-elimination process.

solid-phase reactions.[4] Although the NMR techniques have the advantage that samples can be reused after analysis, they are not suitable for rapid monitoring of functional group transformations. Overall, chromatographic and spectroscopic methods for the detection of on-resin reactions are neither simple nor convenient, and they require relatively long times for analysis.

As part of efforts to develop simple and rapid methods for monitoring functional groups on the resin-supported molecules without the need for specialized instrumentation, colorimetric monitoring tools have been explored.[5] These methods are based on the technique of staining resins that contain specific functional groups. As a result, the progress of a solid-phase reaction can be readily monitored by examining the level of color of the stained resin using eye or microscopic detection. For example, the Kaiser ninhydrin test, widely used for SPPS, has been employed to determine the presence of a primary aliphatic amine on a resin.[6] However, this test is limited to primary aliphatic amines, and it sometimes leads to false positives due to the harsh reaction conditions that are used (1−5 min, 100 °C). The TNBS (2,4,6-trinitrobenzenesulfonic acid) test has also been used to detect on-resin amines, but this method also gives positive results with any resin-bound base.[7]

To improve these old techniques, several colorimetric resin tests, including chloroanil and NF31 tests, have been developed to analyze the extent of completeness of reactions carried out on a polymeric matrix.[8] The detection of resin-bound thiol groups is also important since this functionality

Scheme 1. Synthesis of **1** and **2** Used for the Nondestructive Colorimetric Resin Test



is present in cysteine and other small molecules that are frequently modified with other functional groups. Polymer-supported substances possessing thiol groups can be monitored by using the NF31 procedure.[9] Other functional groups, such as halogens, alcohols, carboxylic acids, and carbonyl groups can be monitored on solid supports by using colorimetric methods.[5]

Although colorimetric resin tests have been successfully employed to monitor the progress of on-resin reactions, most of these methods are limited by the fact that samples can not be reused for the next reactions after resin tests, and as a consequence, the need for repetitive colorimetric resin tests causes a large sample loss. Therefore, the development of a rapid, simple, and nondestructive method to detect the progress of on-resin reactions remains as a significant challenge. Below, we describe the results of a study that has led to the first, resin-reusable, colorimetric method for polymer-bound primary amine, secondary amine, and thiol detection.

The new, nondestructive, colorimetric resin test involves treatment of solid-supported amines and thiols with a dye containing an activated ester group. This leads to the rapid formation of intensely colored beads. Subsequent removal of a colored moiety from solid support after the resin test is performed regenerates the resin for the repetition of the reaction (in case of incomplete reactions) or a subsequent reaction. The molecular system used for this process consists of three parts, including a dye for color detection, an activated

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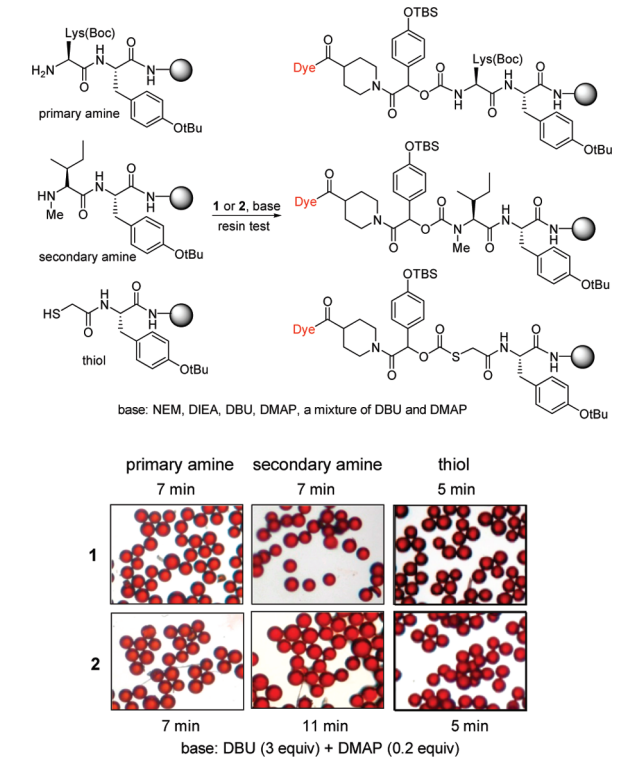

Figure 2. Colorimetric monitoring of polymer-bound amines and thiols using **1** and **2** (NEM: *N*-ethylmorpholine; DBU: 1,8-diazabyclo[5,4,0]undec-7-ene; dye: disperse red 1). Bottom: microscopic images of the resin after reactions of resin-bound amines and thiols (1 equiv) with **1** or **2** (3 equiv) for the given time in the presence of a mixture of DBU (3 equiv) and DMAP (0.2 equiv).

ester for reaction with resin-bound amines and thiols, and a TBS blocked 4-hydroxymandelic acid group to initiate removal of a dye moiety after the color test (Figure 1).[10]

The route for synthesis of **1** and **2**, substances used for the nondestructive colorimetric resin test, was initiated by reaction of disperse red 1 with **3** (Scheme 1). After removal of the Boc group of the product, the liberated amine group was coupled to TBS-protected 4-hydroxymandelic acid (**5**) under HBTU-DIEA conditions to produce **6**. The hydroxyl group in **6** was reacted with *p*-nitrophenyl chloroformate or 1,1′-carbonyldiimidazole to give an activated ester **1** or **2**, respectively. Substances **1** and **2** can be stored as solids and in DMF solutions at room temperature for several weeks.

Initially, we examined whether **1** or **2** is a better reagent for colorimetric monitoring of ploymer-bound amines and thiols. Primary amines, secondary amines, and thiols appended to dipeptides (1 equiv) on a solid support were independently treated with **1** and **2** (3 equiv) in the presence of a single base (3 equiv of NEM, DIEA, DBU, or DMAP) or a mixture of DBU and DMAP for various time periods (1−13 min) at room temperature (Figure 2). After thorough washing of the treated resin, the level of bead coloration was determined using microscopy. Strong, positive responses

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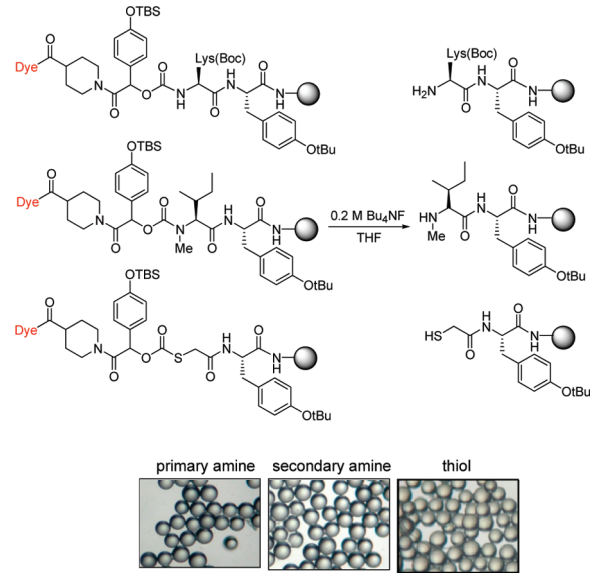

Figure 3. Regeneration of amines and thiols after the color test. Bottom: microscopic images of the resin after reactions of the colored resin with 0.2 M Bu_4NF for 5 min twice at room temperature.

were observed from reactions of **1** with on-resin amines and thiols in the presence of a mixture of DBU (3 equiv) and DMAP (0.2 equiv) for 7 min. However, **2** reacts with secondary amines slower than **1**. Thus, compound **1** was selected for further colorimetric tests.

The results of concentration optimization experiments showed that reactions of polymer-bound amines and thiols with 50 mM **1**, 50 mM DBU, and 10 mM DMAP for 7 min at room temperature gave efficient color tests. Since high-temperature reactions may result in decomposition of resin-bound compounds, making regeneration of compounds after color tests problematic, the resin test was performed at room temperature.

To examine the sensitivity of this new colorimetric method, the resin-bound amine and thiol containing dipeptides (10 μmol) shown in Figure 2 were coupled to 0.9 equiv of Fmoc-Leu. After washing, the resin was treated with **1** (50 mM) and 50 mM DBU−10 mM DMAP for 7 min at room temperature to monitor unreacted amine and thiol groups. The resin was clearly stained by using this procedure, indicating that the minimum detection sensitivity is ca. 1 μmol/g of resin.[11]

Since colorimetric tests of amines and thiols using **1** worked well, we next explored the regeneration of free amines and thiols after performing the color tests. The colored resin, obtained from reactions of resin-bound amines and thiols with **1**, was treated with 0.2 M Bu_4NF in THF for 1−13 min or twice for 5 min at room temperature. These treatments led to the complete removal of the dye moiety from the amine and thiol groups on the resin (Figure 3). On the basis of these results, double cleavage conditions

(11) See Supporting Information.

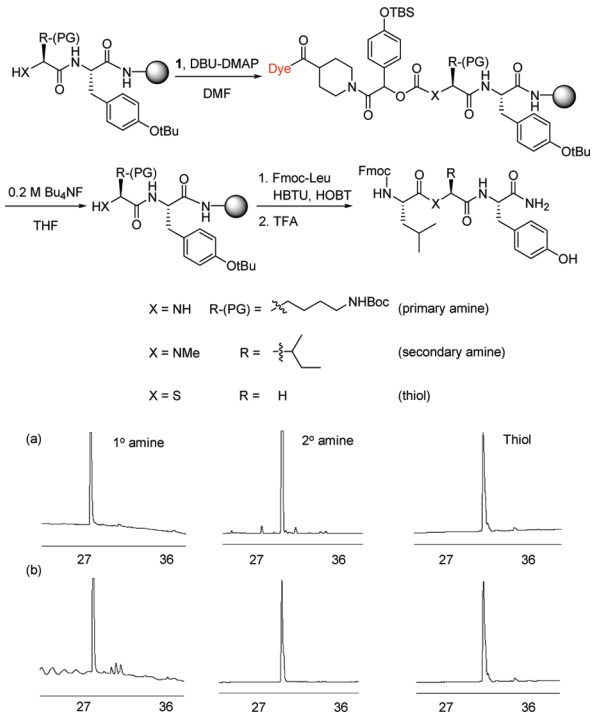

Figure 4. Use of regenerated resins for the next reaction. Bottom: HPLC profiles for (a) tripeptides obtained by using regenerated resins and (b) tripeptides obtained from normal SPPS.

(treatment of the colored resin twice with 0.2 M Bu_4NF for 5 min) were used in further studies. Importantly, the purity of peptides obtained from regenerated resin was quite similar to that obtained without employing the resin test, showing that the resin was successfully recovered.

In practical applications of this new testing method, the resin regenerated after the color test should be used for the next reaction or the repetition of the same reaction. To examine this capability, three polymer-bound dipeptides were first reacted with **1** to stain the resin, and then the dye moiety was removed by the treatment with 0.2 M Bu_4NF. The resulting polymer-bound dipeptides were coupled to Fmoc-Leu to produce polymer-bound tripeptides. HPLC analysis shows that the purity of tripeptides obtained from implementation of this procedure was quite similar to that of tripeptides obtained without intervention of the resin test.

a) FmocNH-Arg-Gln-Lys-NMeIle-Tyr-NH_2
b) FmocNH-Arg-Gln-NMeIle-Lys-Tyr-NH_2
c) FmocNH-Arg-NMeIle-Gln-Lys-Tyr-NH_2
d) Leu-Lys-Leu-Leu-Lys-Lys-Leu-Leu-Lys-Leu-Lys-Gly-linker-$CONHNH_2$
e) Leu-Leu-Lys-Leu-Leu-Lys-Lys-Leu-Leu-Lys-Leu-Lys-Gly-linker-$CONHNH_2$
f) Lys-Leu-Leu-Lys-Leu-Leu-Lys-Lys-Leu-Leu-Lys-Leu-Lys-Gly-linker-$CONHNH_2$
g) Lys-Lys-Leu-Leu-Lys-Leu-Leu-Lys-Lys-Leu-Leu-Lys-Leu-Lys-Gly-linker-$CONHNH_2$
h) Leu-Lys-Lys-Leu-Leu-Lys-Leu-Leu-Lys-Lys-Leu-Leu-Lys-Leu-Lys-Gly-linker-$CONHNH_2$

linker = -$NHCH_2CH_2CH_2CH_2CH_2$-

Figure 5. Sequence of peptides synthesized using the nondestructive colorimetric resin test.

This finding demonstrates the practicability of this nondestructive resin test (Figure 4).

Finally, the new colorimetric resin test was applied for the preparation of several peptides. First, three pentapeptides containing the secondary amide linkage were assembled on the Rink amide resin (Figure 5a−c). In the event that incomplete peptide bond formation takes place during SPPS, the tested resin was regenerated by treatment with 0.2 M Bu_4NF. The recovered resin was then combined with the untested resin for the repetition of the coupling reaction. By following this procedure, we were able to prepare three pentapeptides containing secondary amide linkage in high purities and yields.[11] We were also able to efficiently prepare relatively pure samples of five peptides containing *C*-terminal hydrazide groups by using this method (Figure 5d−h).[11]

In conclusion, we have developed the first, simple, efficient, nondestructive colorimetric resin test to monitor primary amines, secondary amines, and thiols on solid supports. The solid-supported functional groups can be easily detected by this method, and the tested resin can be simply regenerated for the repetition of the same reactions or subsequent reactions. It is expected that this new technique will be applicable in monitoring other nucleophilic functional groups, such as alcohols and hydrazides, since the reagent used in this procedure contains an activated ester.

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Supporting Information Available: Experimental procedure, spectral data, and HPLC chromatograms. This material is available free of charge via the Internet at http://pubs.acs.org.

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