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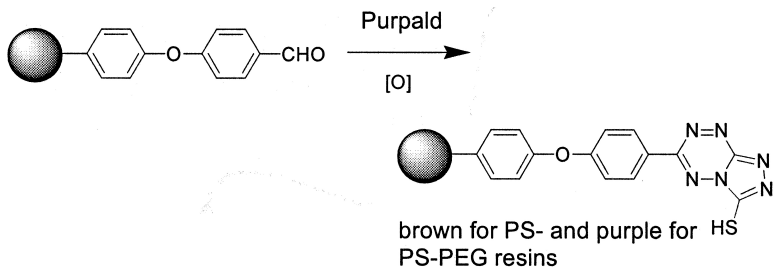

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Color Test for the Detection of Resin-Bound Aldehyde in Solid-Phase Combinatorial Synthesis

Jason J. Cournoyer, Tushar Kshirsagar, Pascal P. Fantauzzi, Gianine M. Figliozzi, Taline Makdessian, and Bing Yan*

ChemRx Advanced Technologies, 385 Oyster Point Boulevard, South San Francisco, California 94080

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We report the development of a sensitive and specific color test for the detection of the presence of resin-bound aldehyde groups using 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (Purpald). Aldehyde resin turns dark-brown to purple after a 5 min reaction followed by a 10 min air oxidation period. Resins that possess other functional groups (i.e., ketone, ester, amide, alcohol, and carboxylic acid) do not change color under the same conditions. The detection limit is 20 μmol/g for polystyrene-based aldehyde resins.

Introduction

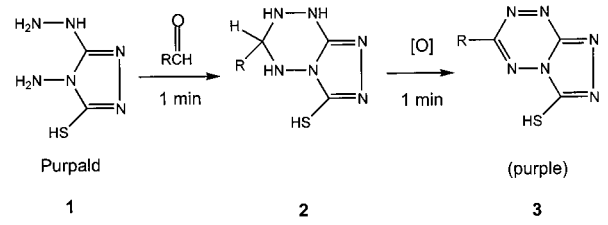
Solid-phase organic synthesis (SPOS) continues to play a dominant role in combinatorial chemistry.[1] However, the optimization of reactions and the development of synthesis protocols remain a formidable task. To develop synthetic methods that will generate high-quality libraries, we are working toward the development of analytical methods to effectively assist in reaction monitoring and chemistry development.

Sensitive identity tests are very useful in reaction optimization. For example, a test that monitors the disappearance of the starting material during the reaction can ensure the reaction completion. There are many color tests for solution-phase organic synthesis, but few tests are available for solid-phase synthesis. Although several identity tests have been used in monitoring solid-phase peptide synthesis, only a handful of methods can be used for monitoring solid-phase organic synthesis.[2−6] Testing reagents used successfully in solution reactions can potentially be used to monitor solid-phase reactions.

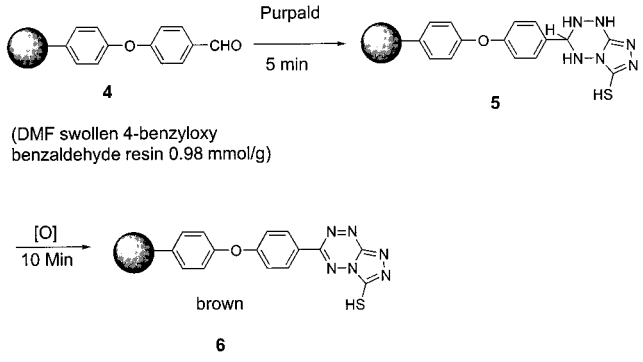
We are interested in methods that specifically detect aldehydes. Traditional solution tests such as the Benedict's, Fehling's, the Tollens', and the Bayer's tests all involve the oxidation of the aldehyde group and the reduction and the precipitation of metal compounds. These tests require that aldehyde compounds be extremely water-soluble, which is less likely in the case of polystyrene-resin-based synthesis. Forming derivatives is another way of detecting aldehyde compounds. Using 2,4-dinitrophenylhydrazine or 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (also known as Purpald, **1**) can produce orange precipitation or intense purple derivatives in solution, respectively. To avoid precipitation formation in solid-phase samples, we chose to study Purpald as a testing reagent. Here, we report our results.

Purpald is reported to specifically react with aldehydes in a 1 N NaOH solution to give **2**.[7,8] Subsequent air oxidation of **2** yielded a purple compound, 6-mercapto-3-substituted-*s*-triazolo-[4,3-b]-*s*-tetrazine **3** (Scheme 1). This reagent can potentially be used as a qualitative test for detecting solid-supported aldehydes. Since reactions on the solid phase tend to behave very differently from those in solution, a method for using Purpald to analyze resin-bound aldehyde needs to be developed. In the following, we report our results on the development of a color test for resin-bound aldehyde compounds, the evaluation of the specificity, the detection limit of this method, and the application of this method in the monitoring of solid-phase reactions.

Scheme 1



Scheme 2



Results and Discussion

Purpald has been used in solution to detect and quantify aldehydes.[7,8] When an aldehyde solution is added dropwise to a Purpald solution in 1 N NaOH, the combined solution turns intensely purple. However, the reaction of gel-type resin-bound aldehydes (Scheme 2) with Purpald is expected to be different. First, resin beads need to be swollen by

* To whom correspondence should be addressed. Phone: 650-228-1293. Fax: 650-228-0138. E-mail: byan@chemrx.com.

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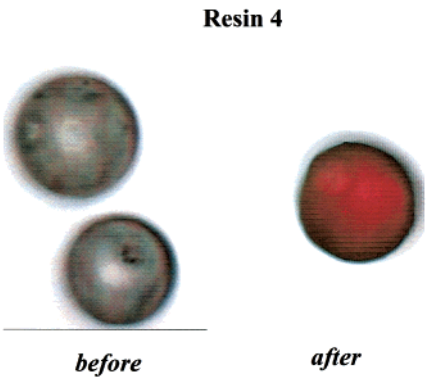


Figure 1. Beads before and after the reaction of resin **4** with Purpald.

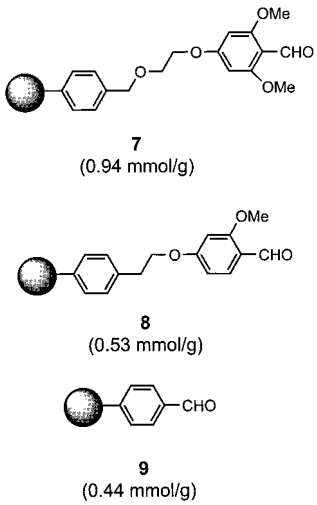


Figure 2. Structures of aldehyde resins used for testing.

appropriate solvents before a reaction can proceed. Second, the reaction between hydrophobic polymeric beads with an aqueous reagent may be slow without a phase-transfer catalyst. Therefore, an investigation of the reaction conditions and the scope and limitation of this method was performed.

Reaction of Purpald with Polystyrene-Based Aldehyde Resins. After 4-benzyloxybenzaldehyde resin **4** (10 mg) was swollen with DMF, the solvent was drained while resin beads still contained DMF. Purpald dissolved in 1 N NaOH solution (1 mL) was added, and the mixture was shaken for 5 min. After being washed, the beads turned dark-brown in 10 min (Figure 1). Reactions of Purpald with three other structurally different aldehyde resins (Figure 2) were also studied. The same reaction conditions were used for these resins, and the intensity of the brown color depended on the loading of resins as well as the aldehyde structure (Figure 2). The order of color intensity in our study was **8** > **9** > **7** (Figure 3). Aldehyde **7**, although it has a high loading, shows less color because of steric hindrance in its structure.

Solvent for the Purpald Test. The polystyrene-based (PS-based) resin is the most popular resin in combinatorial synthesis. Because of the importance of resin swelling of PS resins for the reaction, we carried out reactions in different solvents. Aldehydes bound to dry resin did not react with Purpald dissolved in 1 N NaOH. This indicated that an organic solvent must be used to swell the polystyrene resin

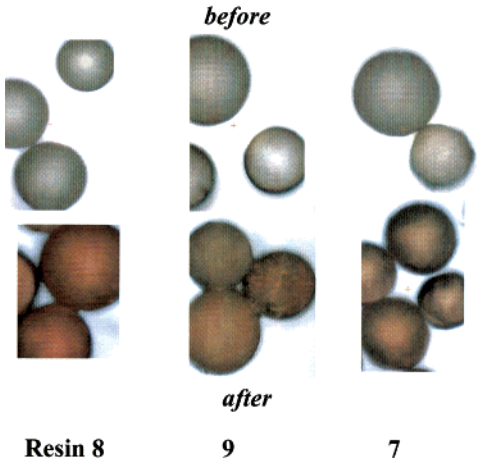


Figure 3. Beads before and after the reaction of resins **7**−**9** with Purpald.

beads before the test. DMF, THF, dichloromethane, methanol, NMP, and dioxane were studied. Because DMF showed the best properties, such as miscibility with the aqueous reagents and the ability to swell resin beads, it was used throughout this investigation.

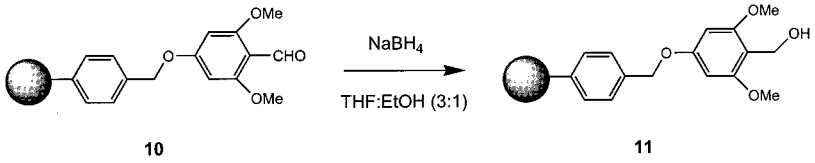
We further tested the requirement for the amount of organic solvent. Swollen beads reacted with the Purpald solution in 1 N NaOH (50 mg/mL) or in a DMF/(1 N NaOH) mixture (1:2 and 1:1). The reaction occurred more rapidly when the DMF swollen beads directly reacted with Purpald in 1 N NaOH. In this case, individual beads are filled with DMF (swollen beads) while the bulk DMF is not present.

Phase-transfer catalyst (PTC) can effectively accelerate reactions on solid phase in aqueous media.[9] In our study, tri-*n*-caprylylmethylammonium chloride (Aliquat)[10] was examined. In the presence of Aliquat, reaction with Purpald produced red beads[10] after a 2 min reaction and a short period of air oxidation. PTC was not used in our final test procedure because the reaction between the swollen beads and the aqueous reagent was sufficiently rapid and the developed brown color was distinct.

Amount of Beads Required for the Test. We carried out the Purpald test using various amounts of resin beads and evaluated the detection of color by eye and by microscope. For visually observing the color change, a total of at least 10 mg of resin beads was required. When an optical microscope was used, a few beads were enough for this test.

Monitoring an Aldehyde Reduction Reaction. Purpald Tests for PS-Aldehyde Resins with Different Loading. The Purpald test was used to monitor a reduction reaction as depicted in Scheme 3. When a 5-fold excess of sodium borohydride was used in this reduction reaction, the PS-resin-bound aldehyde was completely reduced to alcohol in 5 min according to the Purpald test and an independent FTIR study. To further study the required amount of reducing reagent and to test the utility of the Purpald test for resins with various aldehyde loading, we carried out this reduction reaction using various amounts of reducing reagent (0.05- to 2-fold of sodium borohydride) to produce resins with various amounts of aldehyde groups. The relative loading of the aldehyde group on resins was deduced in a two-step

Scheme 3



10 **11**

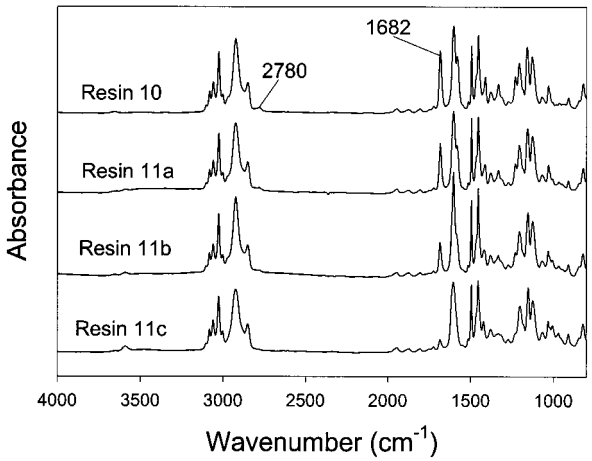


Figure 4. Single-bead FTIR spectra of resins **10** and **11a**−**c**.

Table 1. Relative Aldehyde Loading Estimated by Single-Bead FTIR

resin	peak area ratio, 1682/1944	relative aldehyde loading (%)	calcd aldehyde loading (mmol/g)
10	5.00	100.0	1.13
11a	3.26	75.0	0.84
11b	1.02	38.0	0.43
11c	0.11	1.6	0.02

process: (1) derivatizing the starting PS-aldehyde resin with Fmoc-Gly hydrazide and then cleaving Fmoc to get the absolute loading of resin **10** (1.13 mmol/g); (2) taking IR spectra of the starting resin **10** and the reduction product resins **11a**−**c** to measure the peak areas of their aldehyde carbonyl bands relative to the starting aldehyde resin **10** (Figure 4). Loading results were deduced from their relative IR peak areas and the absolute loading of the starting resin **10**. Results are shown in Table 1. When tested with Purpald, the starting aldehyde resin turned dark-brown, indicating a high loading of aldehyde groups. Resins with an aldehyde loading of 0.84, 0.43, and 0.02 mmol/g showed colors from brown to light-brown (Figure 5). The lowest detectable amount of aldehyde was 20 μmol/g in this study.

Reaction with Resins Having Other Functional Groups. We further investigated the possible interference from other functional groups (Figure 6) with the Purpald test (Figure 7). Resin-bound ketone did not react with Purpald. This result was consistent with what was found in corresponding solution reactions.[7] Resins having ester, acid, amide, alcohol, and amine groups all gave negative results under the same reaction conditions (Table 2).

Reaction of Purpald with PS−**PEG-Based Aldehyde Resins.** Purpald reacted with two PS−PEG resins: TentaGel and NovaGel. In TentaGel the aldehyde groups are located at the end of the long PEG chains. However, in NovaGel, aldehyde groups are located at the polystyrene base while PEG chains are extended as in TentaGel. Compared with

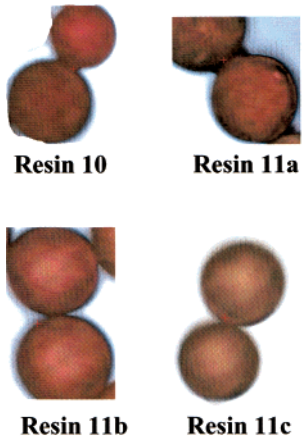


Figure 5. Beads before and after the reaction of resins **10** and **11a**−**c** with Purpald.

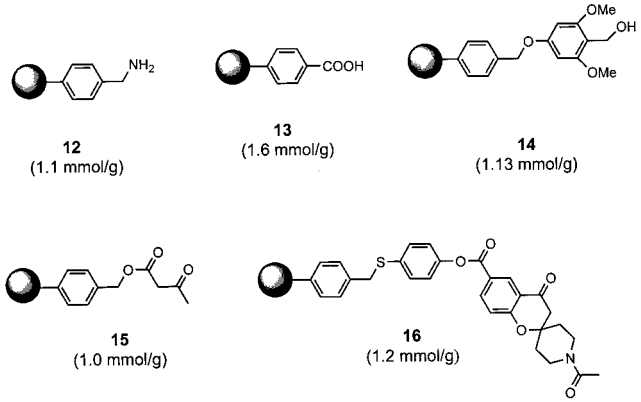


Figure 6. Structures of resins containing various functional groups.

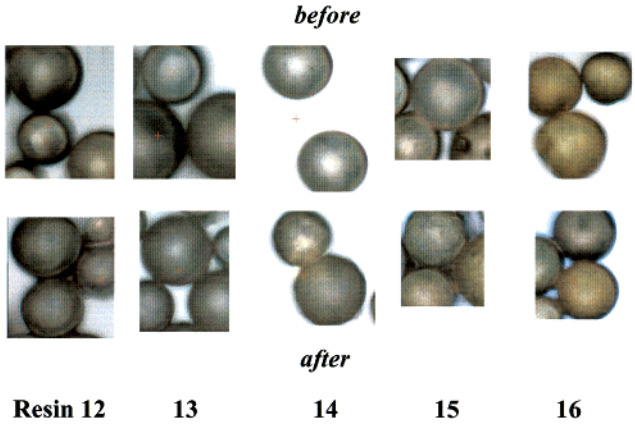


Figure 7. Beads before and after the reaction of resins **12**−**16** with Purpald.

PS-based resins, NovaGel resins offer an improved solubility in water and other polar solvents. TentaGel resins offer both an improved solubility as NovaGel and a better accessibility of the reagent to functional groups. TentaGel reacted with Purpald to give deep-purple color, and NovaGel gave a light-purple color. In comparison, PS-based resins showed a brown

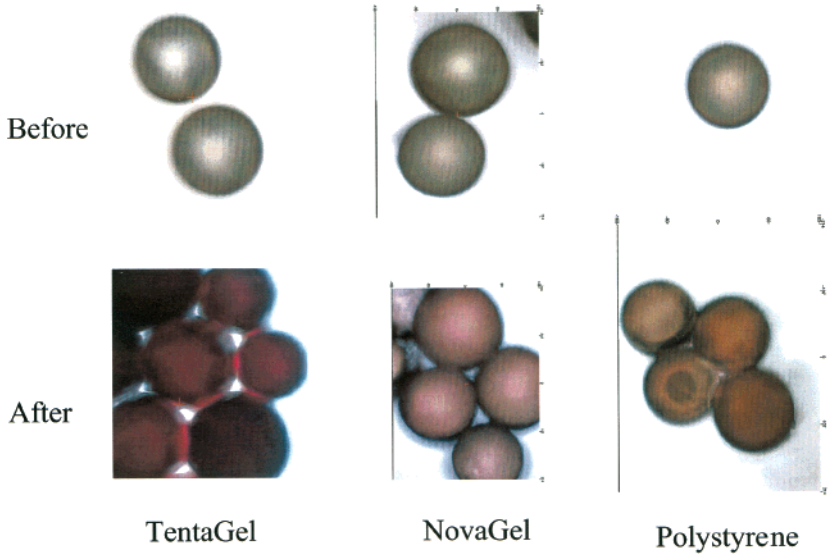


Figure 8. Beads before and after the reaction of TentaGel-based and NovaGel-based aldehyde resins with Purpald in comparison with PS-based aldehyde resin.

Table 2. Purpald Test for Resins with Various Functional Groups

	Purpald test	
resin	positive	negative
9	Y	
12		Y
13		Y
14		Y
15		Y
16		Y

color (Figure 8). We interpret this as both water compatibility and functional group accessibility playing a role in Purpald reactivity.

Concluding Remarks

In the presence of DMF, the reaction between Purpald and resin-bound aldehyde occurred rapidly. The reaction becomes slower when the amount of bulk DMF was increased. The optimal conditions were to react the DMF swollen resin with Purpald solution without the bulk DMF. Water-compatible resins such as NovaGel and TentaGel showed better reactivity with Purpald. Purpald reacted specifically with aldehyde resin and generated dark-brown (or purple for PS−PEG resin) beads after a 5 min reaction and a brief air oxidation. The lowest detection limit was approximately 20 μmol/g for PS-based resin as estimated in this study. Resins bearing other functional groups did not react with Purpald. Purpald was successfully used to monitor the processes of an aldehyde reduction reaction. Purpald is unstable in solution, and the reagent solution should be prepared immediately before the test. In addition, trace amounts of aldehyde from solvents or from the air oxidation of resin-bound alcohol may cause a small color change.

Experimental Section

Materials. All resins were purchased from NovaBiochem (San Diego, CA), and all chemicals and solvents were from Aldrich (Milwaukee, WI).

Single-Bead FTIR Spectroscopy. FTIR spectra were collected on a Nicolet Nexus 670 with continuum microscope, using OMNIC software. The microscope is equipped with a 15× cassegrain objective and a liquid-nitrogen-cooled mercury−cadmium−telluride (MCT) detector. The view mode aided in locating a single bead. The transmission mode was used for the whole bead measurement. Beads flattened with a diamond window (SpectraTech, Shelton, CT) were used for all experiments in transmission mode. A clean diamond window (SpectraTech, Shelton, CT) was used to collect the background spectrum. Data were collected at 4 cm^{-1} resolution, and 32 scans were averaged.

Purpald Test. PS-based or PS−PEG-based aldehyde resin beads (10 mg) were added to a 3 mL filtration tube, and 1 mL of DMF was added to the tube. The tube was capped and shaken in a test tube rack for 5 min on an IKA-Schutter MTS 4 shaker from Janke-Kunkle. The suspension was drained on a vacuum manifold. A solution was prepared by dissolving 50 mg of Purpald in 1 mL of 1 N NaOH. This solution was added to the tube, and the tube was capped and agitated by rotation for 5 min at room temperature on a "rotor-torque" rotator from Cole Parmer Instrument Company. The reaction mixture was drained on a vacuum manifold. Beads were washed with dichloromethane twice (1 mL). In 10 min, the beads changed from a yellow to dark-brown color (positive test for aldehydes).

Reduction of Aldehyde. In this experiment an aldehyde resin **10** (Midwest Biotech no. 20840, lot no. SY03305, 1 g, 1.13 mmol, 1.0 equiv) was suspended in 6 mL of 3:1 anhydrous tetrahydrofuran/ethanol and was subjected to reaction with a 5-fold quantity of sodium borohydride for 5, 10, 20, 40, 80, 160, and 320 min. After the reaction, the resins were repeatedly washed with methanol, dichloromethane, and diethyl ether and were subjected to the Purpald test. In the second experiment, resin **10** was subjected to nine reactions with various amounts of sodium borohydride (0 to 2.0-fold, or 0, 0.057, 0.113, 0.17, 0.226, 0.565, 0.904, 1.13, 2.26 mmol) for 5 min. Resins were washed and tested with Purpald in the same fashion.

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