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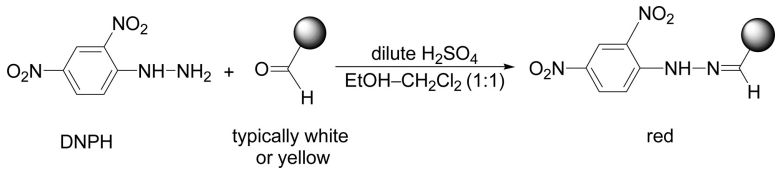

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Colorimetric Monitoring of Solid-Phase Aldehydes Using 2,4-Dinitrophenylhydrazine

Simon K. Shannon and George Barany*

Department of Chemistry, University of Minnesota, 207 Pleasant Street SE, Minneapolis, Minnesota 55455

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A simple and rapid method to achieve colorimetric monitoring of resin-bound aldehydes, based on ambient temperature reaction with 2,4-dinitrophenylhydrazine (DNPH) in the presence of dilute acid, has been developed as an adjunct to solid-phase organic synthesis and combinatorial chemistry. By this test, the presence of aldehydes is indicated by a red to dark-orange appearance, within a minute. Alternatively, resins that are free of aldehydes or in which aldehyde functions have reacted completely retain their original color. The DNPH test was demonstrated for poly(ethylene glycol)−polystyrene (PEG−PS), aminomethyl polystyrene (AMP), cross-linked ethoxylate acrylate resin (CLEAR), and acryloylated *O*,*O*′-bis(2-aminopropyl)poly-(ethylene glycol) (PEGA) supports and gave results visible to the naked eye at levels as low as 18 μmol of aldehyde per gram of resin.

Introduction

Given the fundamental importance[1] of Merrifield's solid-phase method,[2−9] developed originally for peptides[2−9] but subsequently generalized to essentially any class of organic chemical synthesis,[7,8,10−15] it is critical to have reliable and robust companion methods for solid-phase reaction monitoring.[7,8,16−19] The still widely used Kaiser ninhydrin test for amines[20] was but the first example of a solid-phase analytical method, and comparable tests are required for an ever-increasing array of functional groups. Solid-phase reactions should be monitored because incomplete incorporations reduce overall yields and affect purities of products isolated after final cleavage. Classical techniques for following the course of reactions, such as TLC, are clearly not applicable in the solid-phase mode, while on-resin NMR,[21−27] IR,[28−31] MS,[19,32,33] and more recently, electrochemical impedance spectroscopy[34] (ESI) experiments are not straightforward. A viable way to probe progress of a solid-phase reaction is to cleave the intermediate from the linker/support[3,35] and to use classical techniques for characterization and quantitation. However, such approaches may be less than advantageous to the solid-phase chemist because (i) sophisticated equipment might be needed, (ii) not all intermediates are stable to cleavage conditions, (iii) low-load resins may not provide enough product for isolation and characterization, and (iv) the process may entail an unacceptable time delay in developing the information needed to make informed decisions on how to proceed with the synthesis.

Colorimetric and spectroscopic techniques often offer simple, practical tools to qualitatively or quantitatively monitor solid-phase reactions. Support-bound primary and secondary amines have commonly been detected with ninhydrin,[20] bromophenol blue,[36,37] chloranil,[38,39] picric acid,[40,41] and other reagents of interest (recently reviewed[16]). Additional procedures allow the detection of hydroxyls and phenols,[42−48] carboxylic acids,[44,49] thiols,[50] nitro groups,[51] aryl halides,[52] and guanidines.[53] Solid-phase detection of aldehydes can be achieved by a fluorescence-based assay using dansyl hydrazine[54] and by colorimetric methods applying *p*-anisaldehyde[55] or 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (Purpald)[56] (Table 1).

In our hands, the two literature colorimetric tests for aldehydes (i.e., *p*-anisaldehyde and Purpald) present several limitations. For example, *p*-anisaldehyde shows slow reactivity toward unconjugated resin-bound aldehydes, and temperatures near 100 °C are required. Reactions of resin-bound aldehydes with Purpald require aqueous basic media, which is less than desirable when using common hydrophobic solid supports. Consequently, rapid results with Purpald require addition of uncommon phase-transfer catalysts. Moreover, Purpald visualization without the use of a microscope requires at least 10 mg of resin. For both the *p*-anisaldehyde and Purpald tests, it is recommended that the reagent/solutions are prepared freshly before each use.

The present paper describes the use of a classical reagent, 2,4-dinitrophenylhydrazine (**1**, DNPH), to colorimetrically detect aldehydes on common solid supports (Scheme 1).[57] We have found that in the presence of dilute acid, DNPH reacts rapidly with resin-bound aldehydes, leading to formation of a highly conjugated phenylhydrazone derivative (**2**) which can be colorimetrically (red) detected within seconds. Details are provided for preparation of the stable DNPH solution, and the visual sensitivity of this test on several solid support systems and its effectiveness in monitoring model aldehyde conversions are also described.

Results and Discussion

Solid-Phase DNPH Test. The reaction of aldehydes with DNPH to form a colored adduct is textbook introductory organic chemistry.[58,59] We have devised a simple, robust, and sensitive protocol adapting this chemistry to the solid-

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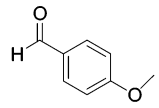
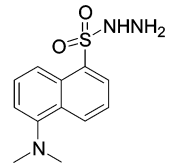
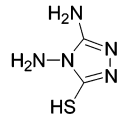
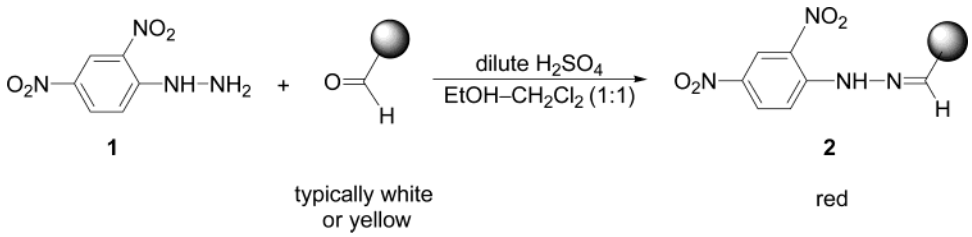
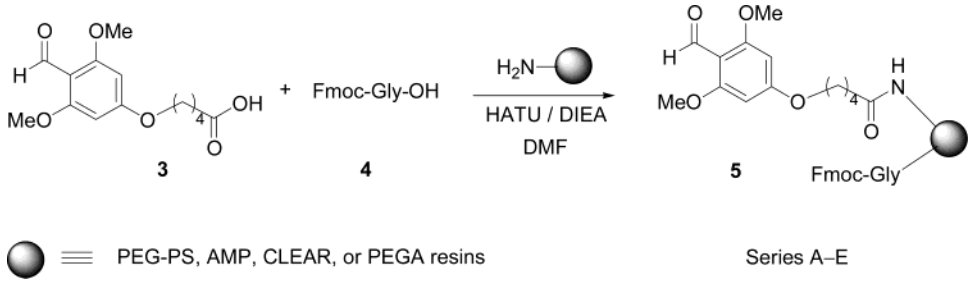
Table 1. Overview of Quantitative and Qualitative Colorimetric Solid-Phase Methods for Monitoring Aldehydes

Reagents and Conditions	Resins Tested	Sensitivity / Comments	Ref.
 *p*-Anisaldehyde–H_2SO_4–HOAc–EtOH (2.5:9:1:88) , 4 min, 100 °C	MBHA (PS) Wang (PS)	7 μmol/g of CHO detected; burgundy to orange on-resin stain; reagent stored for a few days, but freshly prepared reagents is advised	55
 Dansyl hydrazine (2 equiv) in DMF 30 min, 25 °C	Tentagel Formyl (PS)	sensitivity not available; detection of CHO on 3 mg of resin (loading unspecified) reported; *quantitative* technique; based on uptake of dye from supernatant and fluorescence monitoring	54
 0.4 M 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (Purpald) in 1 N aqueous NaOH; 5 min reaction + 10 min for air oxidation, 25 °C	Tentagel NovaGel	20 μmol/g of CHO detected; red to brown on-resin stain; reaction takes place within minutes with phase-transfer catalyst, tri-*n*-caprylylmethylammonium chloride (Aliquat); Purpald is unstable in solution and reagent must be freshly prepared	56

Scheme 1. Reaction of DNPH with Resin-Bound Aldehydes



Scheme 2. Preparation of Aldehyde Resins for Sensitivity Tests



phase mode. A major advantage of our method over previously reported aldehyde tests is that the DNPH reagent/solution is *stable* under ambient conditions and does not need to be freshly prepared before each use. In addition, the test can be carried out on as little as 2 mg of resin, and results are obtained within 1 min of mixing at 25 °C. In the presence of aldehydes, resins immediately take on red to dark-orange appearances. Alternatively, resins that are free of aldehydes, or in which aldehyde functions have reacted completely, retain their original color.

Evaluation of Sensitivity Using PALdehyde[60] Resins (Table 1, Series A−E). Given our interest in backbone amide linker[60] (BAL) anchoring strategies, we decided to couple the aromatic aldehyde 4-formyl-(3,5-dimethoxyphenoxy)valeric acid (PALdehyde) (**3**) onto several common solid supports and thereby determine the lower limit of sensitivity for the DNPH test (Scheme 2). Toward this end, commercially available poly(ethylene glycol)−polystyrene[61,62] (PEG−PS, 0.55 mmol/g), aminomethyl polystyrene[63] (AMP, 0.75 mmol/g), cross-linked ethoxylate acrylate resin[64] (CLEAR,

Table 2. Color Variations upon Treating Various PALdehyde Supports with DNPH[a]

	PEG-PS 0.49 mmol/g[b]	AMP 0.73 mmol/g[b]	CLEAR 0.46 mmol/g[b]	PEGA 0.26 mmol/g[b]
SERIES	% BAL[c] / Intensity	% BAL[c] / Intensity	% BAL[c] / Intensity	% BAL[c] / Intensity
Blank	0	0	0	0
A	5	4	4	3
B	19	24	32	26
C	57	50	49	30
D	70	88	73	77
E	> 99	> 99	> 99	> 95

[a] All photographs were taken using a Canon Powershot G1 digital camera. Captured images represent the bottoms of 12 × 75-mm test tubes containing ~10 mg of aldehyde resin. [b] Actual loadings were calculated by first quantitatively loading commercially available resins with Fmoc-Gly-OH (3 equiv), as mediated by HOBt/DIPCDI (3 equiv each) in DMF. A resin aliquot was then treated with piperidine−DMF (1:1), and the piperidine−dibenzofulvene adduct from Fmoc cleavage was quantified by absorption at 301 nm.[6,9] [c] Partially substituted PALdehyde resins were prepared by mixing PALdehyde and Fmoc-Gly-OH in ratios of 1:99 (series A), 1:3 (series B), 1:1 (series C), 3:1 (series D), and 99:1 (series E) and coupling to the respective resins using HATU/DIEA protocols. Actual loadings were then determined by the protocol of note *b*, above (further information in Supporting Tables 1 and 2).

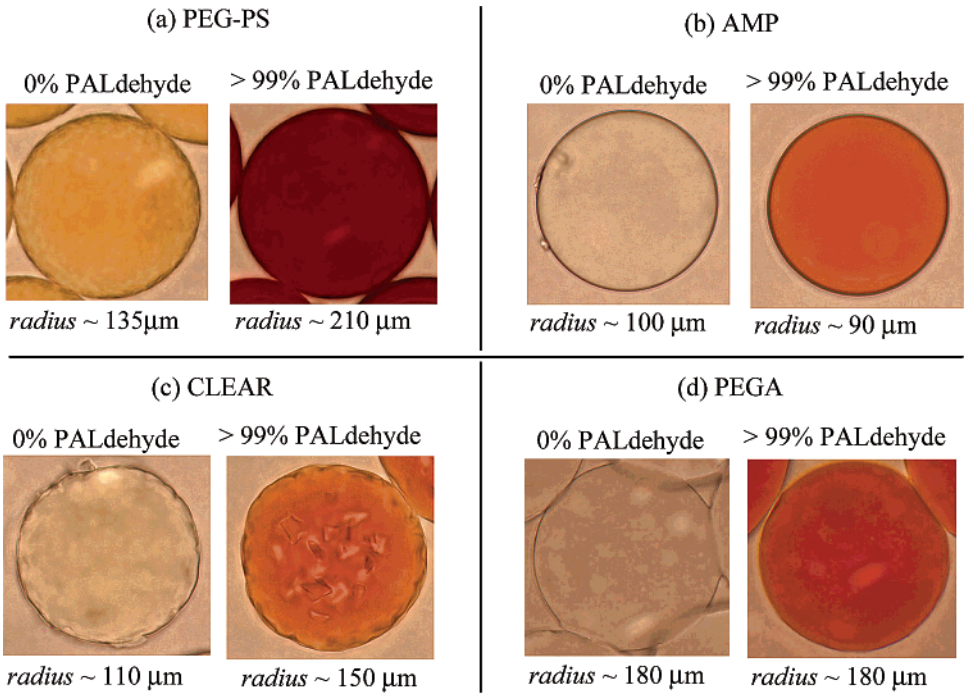


Figure 1. Magnified photographs showing the shapes and textures of (a) PEG−PS, (b) AMP, (c) CLEAR, and (d) PEGA loaded with 0% (left) and >99% (right) PALdehyde, and swollen in CH_2Cl_2, after carrying out DNPH tests for aldehyde content. Working magnification, 20×. Image sizes were reduced to fit the page.

0.66 mmol/g), and acryloylated *O*,*O*′-bis(2-aminopropyl)-poly(ethylene glycol)[65,66] (PEGA, 0.30 mmol/g) resins were intentionally loaded with increasing amounts of **3**. Several mixtures of **3** and Fmoc-Gly-OH (**4**), in mole ratios of 1:99 (series A), 1:3 (series B), 1:1 (series C), 3:1 (series D), and 99:1 (series E) [3 equiv combined carboxylic acid function], respectively, were prepared and coupled to resins as mediated by *N*-[(dimethylamino)-1*H*-1,2,3-triazolo-[4,5-*b*]pyridin-1-yl-methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (HATU) (3 equiv) and *N*,*N*-diisopropylethylamine (DIEA) (3 equiv). "Blanks" were prepared to represent negative controls by mixing PALdehyde with the respective

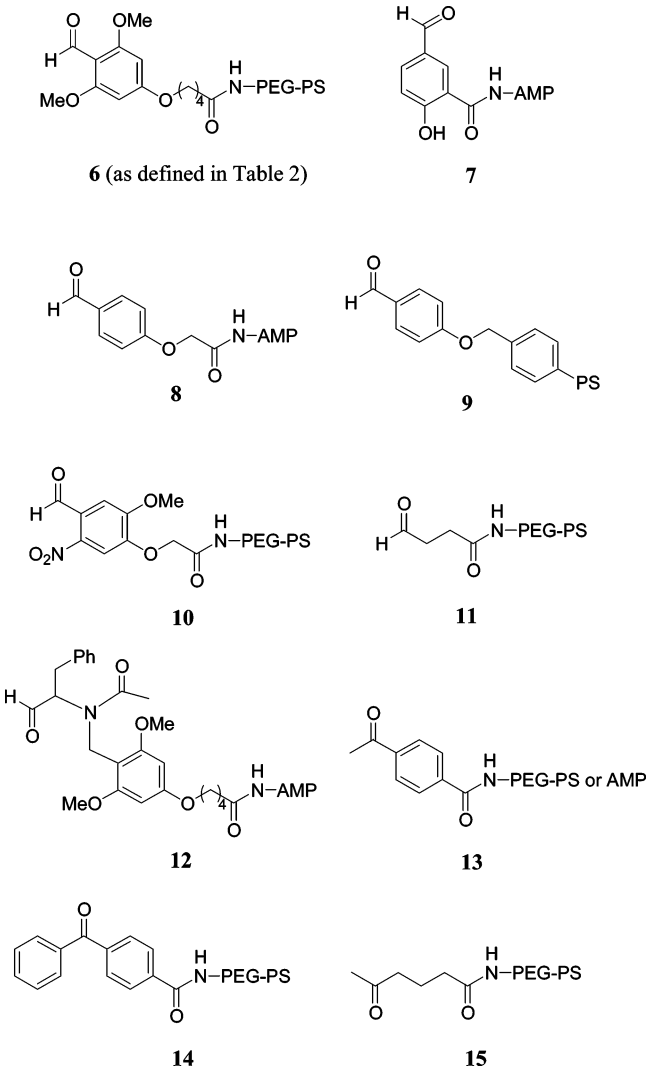


Figure 2. Structures of various resin-bound aldehydes and ketones subjected to the DNPH test.

resins in *N*,*N*-dimethylformamide (DMF) *without* coupling reagents. Actual loadings of resin-bound PALdehyde were calculated indirectly by treating 10 mg of resin with piperidine−DMF (1:1) to remove Fmoc, followed by quantitation of the resulting piperidine−dibenzofulvene adduct at 301 nm using an ultraviolet spectrometer.[6,9]

The blank and series A−E (**5**) for each resin were subjected to the DNPH test, and the resultant beads were photographed (Table 2 and Figure 1). As the loading of aldehyde increased, significant changes in color were observed. At the maximum level (series E), the intensity of red staining appeared to increase in the order of PEGA < CLEAR < PEG−PS < AMP. At the minimal level (series A), all resins gave positive tests, although the intensity for PEGA was weakest. CLEAR, PEG-PS, and AMP showed relatively the same color intensities with each series level. Visual detection of aldehydes with the naked eye was possible at levels as low as 3% (i.e., a sensitivity of 18 μmol/g). Magnified photographic images revealed obvious changes in color as well as differences in resin properties (Figure 1).

Scope and Limitations. The DNPH test was performed further on various carbonyl resins related to our research (Figure 2). Resin-bound aromatic aldehydes with activating

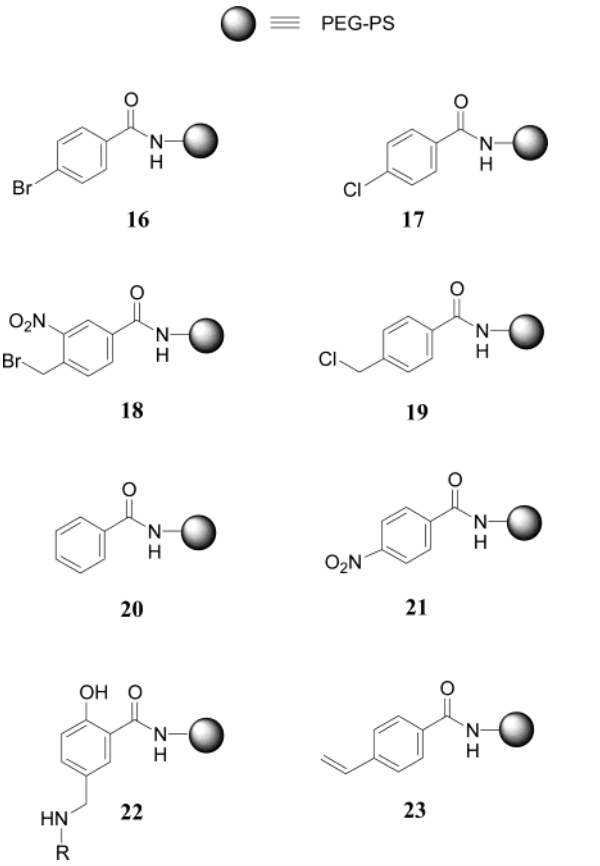


Figure 3. Structures of various resin-bound functional groups subjected to DNPH for interference tests. All of the supports shown tested negative, meaning that they did not interfere with DNPH.

groups [methoxy (**6**), hydroxy (**7**)] in the ortho or para positions showed strong red intensities. On-resin unsubstituted *p*-alkoxybenzaldehydes, such as resins **8** and **9**, or aromatic aldehydes with deactivating groups, such as the nitro functionality in resin **10**, still gave good reactions, but slightly weaker intensities were observed. Aliphatic aldehyde resins **11** and **12** gave relatively the lowest, but still noticeable, changes from their original resins' color. Finally, the DNPH test can be applied successfully to a range of different supports (i.e., PEG−PS, AMP, Wang, CLEAR or PEGA), and there is no significant change in color and intensity as a function of support.

We have also examined three representative ketones (**13**−**15**) using otherwise the identical procedure. We found that color was developed with ketone **13**, albeit at a modest level. Qualitatively, the intensity from ketone **13** was similar to that of aliphatic aldehydes. It took ∼30 min at 25 °C before any color change occurred on ketones **14** and **15**. Therefore, our preliminary conclusion is that although the method does report the presence or absence of ketones, it does not have the sensitivity level for rapid routine application.

In the course of our research, the DNPH test has been a useful tool for monitoring the progress of a number of solid-phase conversions, including (a) attachment of aldehyde linkers to solid supports, (b) reduction of Weinreb amides, and (c) monitoring of reductive aminations (see Supporting Information, Schemes 1−3). Furthermore, interference from other functional groups [e.g., aryl halide, benzyl halide, amide, nitro, phenolic, and vinyl (compounds **16**−**23**, Figure

3)] is negligible. Finally, it must be pointed out that the DNPH solution itself is orange; therefore, thorough rinsing of resins with MeOH is required to avoid false positives.

Conclusions

A simple, sensitive, and rapid method for the colorimetric monitoring of aldehydes on several solid supports has been thoroughly investigated. The DNPH test introduced here presents several advantages over previously described solid-phase colorimetric aldehyde tests. Results are obtained within 1 min at 25 °C, allowing detection of aldehydes sensitively (18 μmol/g) with as little as 2 mg of aldehyde resin. The DNPH test solution is stable at ambient temperatures and can be used with confidence after several months of storage. Reactions of DNPH with other resin-bound ketones as well as new methods to quantitate solid-phase aldehydes are currently under investigation.

Experimental Section

General Procedures. Solution and solid-phase reactions, as well as resin washes, were carried out at 25 °C, unless indicated otherwise. Polymer-supported reactions were performed in plastic syringes (3 mL) fitted with polypropylene frits, then were rotated on an EStem Electrothermal Reacto-Station RS 6000 orbital shaker. DNPH, concentrated sulfuric acid (H_2SO_4), and all solvents were reagent grade from Aldrich (Milwaukee, WI). Resins and specialty reagents were obtained as follows: PEG-PS resin (0.55 mmol NH_2/g) and PALdehyde were from PE Biosystems (Framingham, MA); Boc-Gly-OH and HATU were from Advanced ChemTech (Louisville, KY); AMP resin (0.75 mmol NH_2/g) was from AminoTech (Ottawa, Ontario); CLEAR resin (0.66 mmol NH_2/g) was from Peptides International, Inc. (Louisville, KY); and PEGA resin (0.40 mmol NH_2/g) was from Polymer Laboratories (Amherst, MA). CH_2Cl_2 was freshly distilled from anhydrous calcium hydride. Ultraviolet/visible spectroscopy was performed on a Beckman DU 650 spectrophotometer. Photographs in Table 2 were taken using a Canon Powershot G1 digital camera (Lake Success, NY). Magnified photographs in Figure 1 were taken using a Zeiss Axio Plan 2 microscope (Thornwood, NY) equipped with a Diagnostics Instruments, Inc. spot camera (Sterling Heights, MI).

Preparation of DNPH Reagent/Solution and Solid-Phase Protocol To Test for Aldehydes. The DNPH reagent/solution was prepared by first dissolving 2,4-dinitrophenylhydrazine (100 mg) in concentrated H_2SO_4 (0.5 mL) and then adding this solution slowly, with stirring over 1 min, to H_2O−EtOH (1:10, 7.7 mL). This DNPH reagent/solution can be stored under ambient conditions for a minimum of several months [no drop-off in performance at the end of this time]. Approximately 2 mg of resin was transferred to a clean, dry test tube, and CH_2Cl_2 was added dropwise until the resin swelled and was immersed completely in CH_2Cl_2. Next, three drops of the DNPH solution were added to the test resin, and the resulting red-orange suspension was agitated on a vortex mixer for 1 min at 25 °C. [Typically, results are seen immediately, but the rinsing cycle which follows should be used to get the most reliable results.] The suspension was then diluted with MeOH (2 mL) and decanted for several cycles (in each cycle, the resin sinks to the bottom) until the decanted MeOH solution was nearly colorless. A positive test is indicated by a red to dark-orange resin appearance. For resins that are free of aldehydes, or in which aldehyde functions have reacted completely, the original resin's color is retained.

Preparation of PALdehyde Resins (Table 2, Series A−E) for Sensitivity Tests. PEG−PS (0.55 mmol NH_2/g), AMP (0.75 mmol NH_2/g), CLEAR (0.66 mmol NH_2/g), and PEGA (0.30 mmol NH_2 per g) resins (∼50−150 mg for each series) were swollen in CH_2Cl_2 (2.5 mL, 5 min) and washed thoroughly with DMF−DIEA (4:1, 5 × 2.5 mL). PALdehyde and Fmoc-Gly-OH were mixed in separate ratios of 1:99, 1:3, 1:1, 3:1, and 99:1 (3 equiv combined carboxylic acid function) with HATU/DIEA (3 equiv each), and dissolved in DMF (2.5 mL). Supporting Table 1 lists the exact amounts of resin, PALdehyde, Fmoc-Gly-OH, HATU, and DIEA used to prepare each series in Table 2. The resultant solutions were added to the resins and rotated on an orbital shaker for 24 h at 25 °C to provide series A−E for each resin set [see text for description of "blank"]. The partially substituted aldehyde resins were then washed with DMF (5 × 2.5 mL), MeOH (5 × 2.5 mL), and CH_2Cl_2 (5 × 2.5 mL), and dried (2 mm, overnight in a desiccator). Actual loadings of resin-bound PALdehyde were calculated indirectly by treating 10 mg of resin with piperidine−DMF (1:1) to remove Fmoc, followed by quantitation of the resulting piperidine−dibenzofulvene adduct at 301 nm using an ultraviolet spectrometer.[6,9] (see note *b* from Table 2; see also Supporting Tables 1 and 2).

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Supporting Information Available. Supporting Table 1 lists the exact amounts of resin, PALdehyde, Fmoc-Gly-OH, HATU, and DIEA used to prepare each series in Table 2. Supporting Table 2 includes the resin used, amount of resin quantified, average absorbance, calculated loading, percent NH_2, and percent CHO for each resin series. Supporting Schemes 1−3 display model reactions monitored by the DNPH test. This material is available free of charge via the Internet at http://pubs.acs.org.

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