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A useful and sensitive color test to monitor aldehydes on solid-phase

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Abstract—A rapid and sensitive color test to monitor the presence of aldehydes on solid-phase resins has been developed.

During the last decade, the solid-phase methodology first developed by Merrifield[1] for the preparation of peptides has been rapidly and extensively applied for the preparation of the so-called small organic molecules.[2] The main advantages of the solid-phase approach are: (i) simplified manipulations, because all processes are carried out in just one reactor; (ii) simplified work up, because the excess of reagents and soluble side products can be removed by filtration and washing; (iii) reagent excess can drive reactions to completion; and (iv) automation and/or parallelization can be implemented. On the other hand, it also presents some drawbacks. The control of the progress of the reaction is very often not carried out. This, together with lack of purification and characterization of the intermediates, frequently favors the obtention of complex final mixtures. Therefore, there is a need for colorimetric tests for the identification of organic functional groups for monitoring of the completeness of reactions. Besides the ninhydrin[3] and the bromophenol blue[4] tests for the determination of free primary amines, others have been developed for other functions such as the hydroxyl[5] and carboxylic acid[6] groups. To the best of our knowledge, no test is available for the determination of aldehydes. Aldehydes, in addition to being versatile functional groups for the construction of complex molecules, are the base of a broad family of functionalized solid supports. Thus, following the appearance in the literature of the 5-(3,5-dimethoxy-4-formyl)valeryl (BAL) resin[7] for the solid-phase preparation of carboxamides and sulfonamides, other similar resins lacking one[8] or both methoxy[9] groups have been described and commercialized.[10] In these resins the incorporation of the first building block is usually done through an amino function by reductive amination.[7b] It is important to control the progress of this reaction because poor incorporations will lead to a decreased overall yield, as well as the possible formation of impurities arising from the posterior reaction of any unreacted aldehyde.

Bearing this in mind, we concentrated our efforts on the adaptation to the solid-phase mode of one of the methods used for the detection of aldehydes in TLC. From those described in the literature,[11] the one which uses *p*-anisaldehyde in the presence of a strong acid appeared to be, in principle, more suitable.[12] This method serves to detect both aromatic and aliphatic aldehydes. In our protocol, instead of perchloric acid a mixture of sulfuric and acetic acids is used.

To determine the sensitivity of the method, several BAL resins were prepared by limited incorporation of a BAL handle to an MBHA resin. Depending on the amount of aldehyde present on the resin, the resin with the *p*-anisaldehyde solution becomes orange to red after treatment (see Table 1 and Fig. 1).

Table 1. Assessment of the test sensitivity with resin samples containing different amounts of aldehyde groups

Sample	CHO content (%)	Color
A	0	Colorless
B	1	Pale orange
C	6	Light orange
D	30	Orange
E	54	Red
F	98	Burgundy

Abbreviations: BAL, backbone amide linker; DIEA, *N*,*N*-diisopropylethylamine; DMF, *N*,*N*-dimethylformamide; Fmoc, 9-fluorenylmethoxycarbonyl; TBTU, *N*-[(1*H*-benzotriazol-1-yl)(dimethylamino)-methylene]-*N*-methylmethanaminium tetrafluoroborate *N*-oxide.

Keywords: analytical method; colorimetric assay; combinatorial chemistry; high-throughput synthesis.

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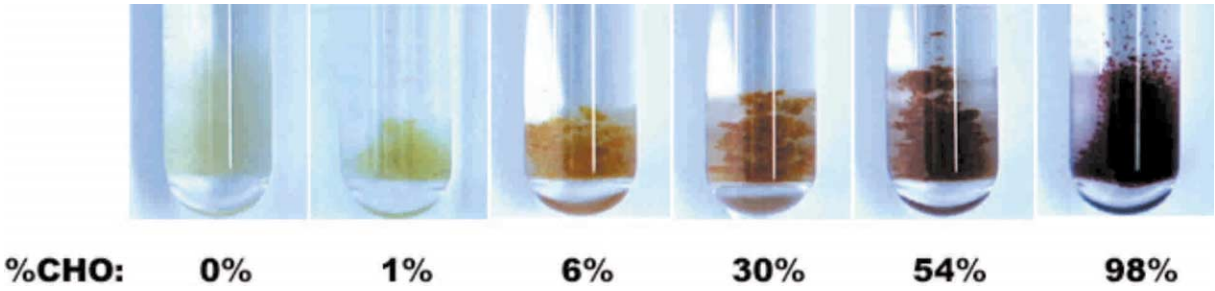

Figure 1.

In order to check the compatibility of this test with more acid-labile resins, such as Wang and chlorotrityl resins, the BAL handle was incorporated into both resins through an ester bond and samples were submitted to the *p*-anisaldehyde test. Using the Wang resin similar results were obtained; however, in the case of the chlorotrityl resins, the solution also took on the color indicating, as it might be anticipated, cleavage of the BAL handle from the resin.

Furthermore, the *p*-anisaldehyde test is compatible with the presence of other functions such as amine or hydroxyl groups.

In conclusion, a rapid and sensitive test for the detection of aldehydes on the solid-phase has been developed.

Experimental protocols

Preparation of resins. With the exception of resin **A**, which was simply MBHA resin (0.7 mmol/g), all the resins were prepared from MBHA resin according to previously described procedures by TBTU–DIEA coupling using the following equivalents of BAL handle: **B**: 0.01 equiv. and **C**: 0.1 equiv. (both for 16 h); **D**: 0.5 equiv., **E**: 1.0 equiv. and **F**: 2.0 equiv. (each for 30 min).

Determination of the CHO group content was carried out by incorporation of Fmoc-Gly-OH on a sample of the resin, removal of the Fmoc group by piperidine–DMF (2:8), and UV spectroscopic determination of the dibenzofulvene adduct at 300 nm.[13]

Solution test. Ethanol (88 mL), sulfuric acid (9 mL), acetic acid (1 mL), and *p*-anisaldehyde (2.55 mL) were mixed and the resulting solution was stored at 4°C. The solution can be kept for a few days, but the use of freshly prepared solution is advisable.

Experimental procedure for the solid-phase test of aldehydes. A few methanol-washed beads of the resin were placed into a small test tube. 300 μl of the test solution were added and the tube was heated at 110°C for 4 min (a blank control test using only MBHA resin is advisable).

If no free CHO are present, the beads should appear colorless, as does the blank. If color appears, CHO groups are present on the resin (see Table 1). The use of a microscope is recommended for low aldehyde content.

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