Pergamon

SCIENCE DIRECT®

Tetrahedron Letters 44 (2003) 4319–4320

TETRAHEDRON LETTERS

Detection of primary aromatic amines on solid phase

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Received 4 January 2003; revised 16 April 2003; accepted 16 April 2003

Abstract—A method for the detection of aromatic amines on the solid support by using chloranil has been developed. This test can detect as little as less than 5 μmol g^{-1} of primary aromatic amines attached to the resin.

Since 1970, the Kaiser test has been used extensively to monitor the progress of amide bond formation during solid phase peptide synthesis. This test is based on the specific color reaction of ninhydrin with a primary aliphatic amino group.[1] The presence of the secondary amino group of proline can be detected using 2,3,5,6-tetrachlorobenzoquinone (chloranil) and acetaldehyde.[2] These tests have been used to monitor peptide synthesis both on solid phase and in solution. In small molecule combinatorial chemistry, a need exists for the development of simple but sensitive tests to monitor the completion of specific reactions on solid support. We have recently reported the development of a novel, encoded 'one-bead one-compound' small molecule library method.[3] We have chosen nitro-phenylalanine as the scaffolding. Aromatic nitro compounds are convenient solid phase synthesis building blocks because the aromatic nitro-group can easily be reduced by tin(II) chloride to a primary aromatic amine, which can be transformed further either by alkylation with alkyl halides, reductive alkylation with aldehydes, or acylation with acids, anhydrides and chlorides.[4] However, there is no reliable test that has been reported to monitor the progress of these reactions.

Condensation of aniline with benzoquinone has been used for the preparation of dyes since the second half of the nineteenth century[5] and has been recently employed in the synthesis of indolocarbazole quinones.[6] We have exploited this reaction (Fig. 1) as a simple and sensitive method to detect secondary amines during our small molecule library synthesis.

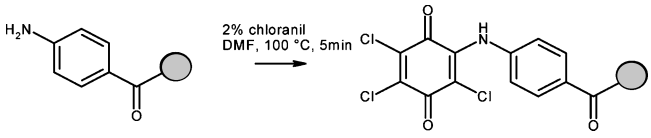

Figure 1. Reaction of resin bound 4-aminobenzoic acid (Abz) with chloranil.

To evaluate this color reaction with a number of primary aromatic amines,[7] we mixed the amines with 2% solution of chloranil in dimethylformamide. All amines tested produced an intensive brown or violet color within 5 min at room temperature, whereas the solution of chloranil remained bright yellow. To increase sensitivity, we heated the sample to 100°C, which was necessary for detection of aromatic amines on solid support.

To determine the sensitivity of the chloranil test, we prepared four TentaGel S NH_2 resins (Rapp Polymere, Tubingen, Germany) with minute quantities (26, 13, 6.5 and 2.6 μmol g^{-1}) of 2-aminobenzoic acid (2-Abz), 3-aminobenzoic acid (3-Abz) and N^{α}-acetyl-4-aminophenylalanine (Ac-Phe(4-NH_2)). Reactants used were Fmoc-3-Abz-OH, Boc-2-Abz-OH, Fmoc-Phe(4-NHBoc)-OH, diisopropylcarbodiimide and *N*-hydroxybenzotriazole. Free amino groups and N^{α} of Phe(4-NH_2) were capped with acetic anhydride and the protecting groups, Fmoc and Boc, were removed using 20% piperidine and trifluoroacetic acid, respectively. The chloranil test was then performed on all bead-samples using the procedure discussed below.

Keywords: solid phase synthesis; aromatic amine detection; aniline detection.

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0040-4039/03/$ - see front matter
doi:10.1016/S0040-4039(03)00984-5

Table 1. Detection of resin bound aromatic amines with chloranil (++ intense color, + less intense color, ± not clear)

Resin subst. [μmol g^{-1}]	26	13	6.5	2.6
2-Abz	++	+	+	±
3-Abz	++	+	+	±
Ac-Phe(4-NH_2)	++	++	+	+

A minute sample of the resin (approx. 1 mg) was transferred to a small glass test-tube, washed with ethanol, and one drop of a 2% solution of chloranil in dimethylformamide was added. The mixture was then heated to 100°C for 5 min in a heating block. In the presence of primary aromatic amino group, the resin turned red. Staining intensity was monitored visually on a white background and compared with both the other samples and the 100% acetylated TentaGel resin as a negative control. Semi-quantitative results are summarized in Table 1. The color intensity is slightly affected by the position of amino group due to steric hindrance (*ortho*-<*meta*-<*para*-).

The remaining resin samples were alkylated with propylaldehyde using a reductive alkylation procedure.[8] The chloranil test was then performed on these resins, and in all cases was found to be negative. The chloranil reaction is specific to primary aromatic amines attached to solid phase. In our experience, secondary and tertiary aromatic amines, as well as pyridine and pyrimidine moieties, all tested negative under these conditions.

In conclusion, the chloranil test described in this report is simple, rapid, sensitive, and can be used to monitor reactions with primary aromatic amines during solid phase synthesis.

Acknowledgements

This work was supported by NIH grant number R33CA-86364. We would like to thank Alan Lehman for reading our manuscript.

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