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A sensitive visual test for detection of OH groups on resin

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Abstract

The presence of an OH group on a resin can be visualized with a colourimetric test based on the anchoring of a carboxylic dye on a chlorotriazine covalently linked to the hydroxyl group. 2,4,6-Trichloro-[1,3,5]-triazine (TCT) and Alizarin R (AliR) colour OH containing beads as deep yellow–red globes. © 2000 Elsevier Science Ltd. All rights reserved.

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The enormous development of synthesis on solid support has been one of the major innovative contributions to organic and medicinal chemistry over the last decade.[1] Notwithstanding the large popularity won by this method, a certain resistance against SPOS still occurs in the community of organic chemists. One of the possible reasons is the lack of rapid and simple tests to evaluate the progress of a reaction on the beads. Non-destructive methods such as FT-IR and gel-phase NMR give useful information on the chemical structure of the beads.[1a] Mass analysis has also been demonstrated as a useful analytical method for bead analysis by using MALDI–TOF MS.[1a,c] However, none of these techniques are as immediate as the classical TLC or GC analysis.

Practicle alternatives to TLC are the colour tests such as the Ellman test for thiols,[2] the Kaiser test for primary amines,[3] the chloranil test for secondary versus tertiary amines[4] and other tests for amines such as the NPIT,[1d] TNBS[5a] and NF31 tests.[5b] Although very effective, these methods are exclusively for thiols and amines.[6]

Recently, we described the use of [1,3,5]-triazine as a useful template to link a carboxylic acid to a NH_2 terminal resin.[7] As it is known that 2,4,6-trichloro-[1,3,5]-triazine (TCT) reacts selectively at one position with alcohols[8] and that the resulting 2-alkoxy-4,6-dichloro-[1,3,5]-triazine is still able to react rapidly with a carboxylic acid at another position,[9] we thought that TCT could be an ideal linker to anchor a coloured molecule to an OH group present on a resin.

Thus, we prepared the dipeptide *N*-Fmoc-Ser-Gly on a Wang resin coupling *N*-Fmoc-Ser-OH with the linked Gly-NH_2. The coupling was carried out using 4-(4,6-dimethoxy-[1,3,5]-triazin-2-yl)-4-methyl-morpholinium chloride (DMTMM) which is known to selectively form the amide

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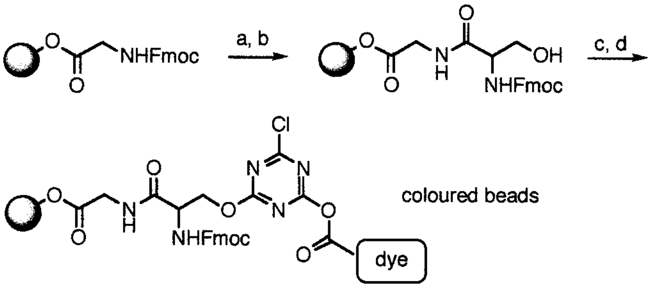
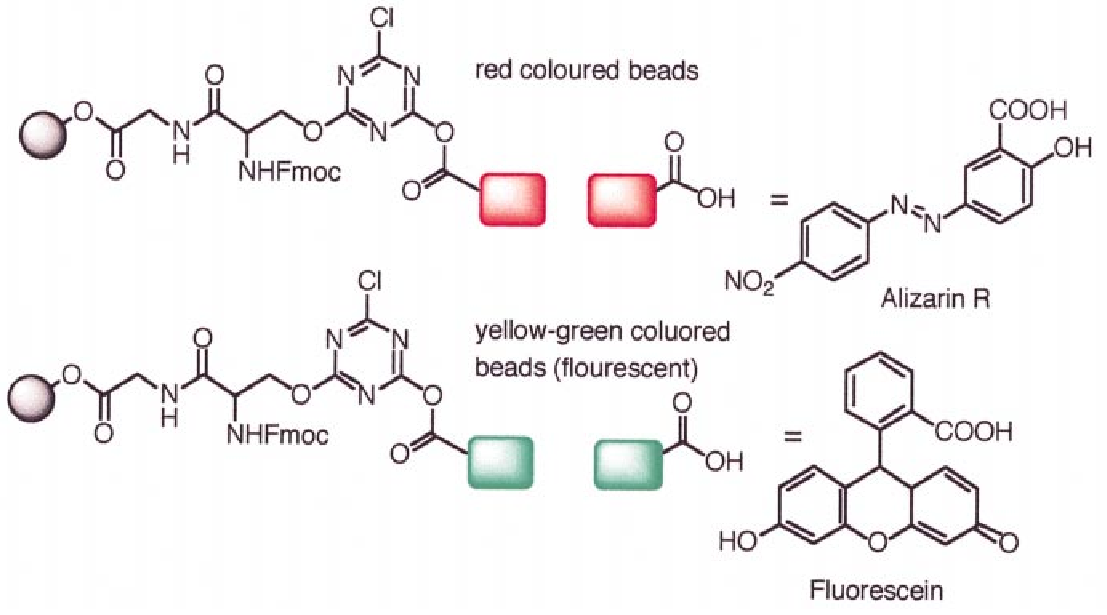
bond without affecting the free OH group on the serine residue.[10] The presence of the peptide on the resin was confirmed by the negative ninhydrin test on the beads after the coupling and by the quantitative determination of the presence of the Fmoc after deprotection with piperidine (Scheme 1).[1d]



Scheme 1. (a) Piperidine, DMF 25% 20 min, rt; (b) *N*-Fmoc-Ser-OH, DMTMM, NMM, NMP, 0.5 h, rt; (c) TCT, NMM, THF, 10 min, rt; (d) Alizarin R (or fluorescein), NMM, THF 5 min, rt

Thus, a few beads of the resin were taken from the reactor, transferred to a test tube and reacted with TCT and *N*-methyl morpholine (NMM) in THF. After 10 min at room temperature the beads were washed several times with DMF and THF and a carboxylic acid containing dye was reacted in THF in the presence of NMM. After a few minutes the beads were rinsed several times with DMF until the solution was no longer coloured. As carboxylic acid dyes we chose commercially available Alizarin R (AliR) or Fluorescein (as the sodium salt) and we observed the resin as red or yellow–green beads, respectively (Scheme 2).



Scheme 2.

In both cases the colours were observed under a microscope (10 times magnification is sufficient, see Fig. 1a). A fully positive test was also observed directly in the test tube as the red or the yellow–green beads are visible to the naked eye (see Fig. 1b).[11]

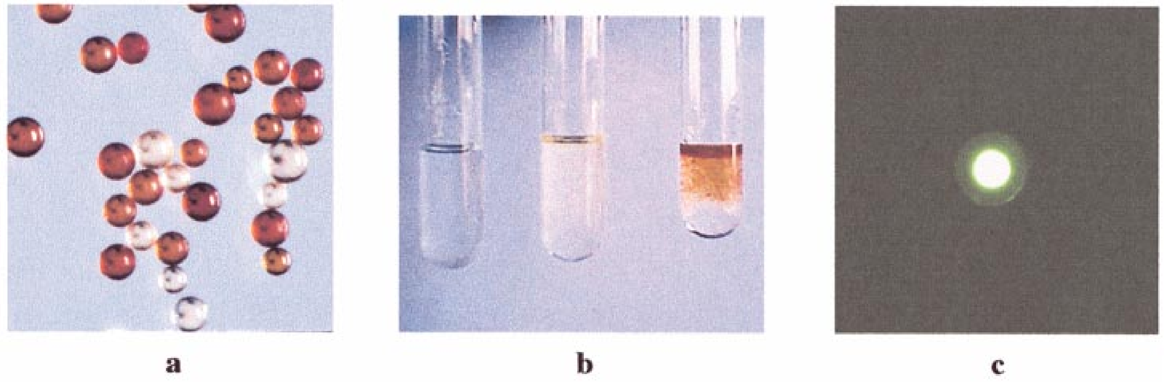
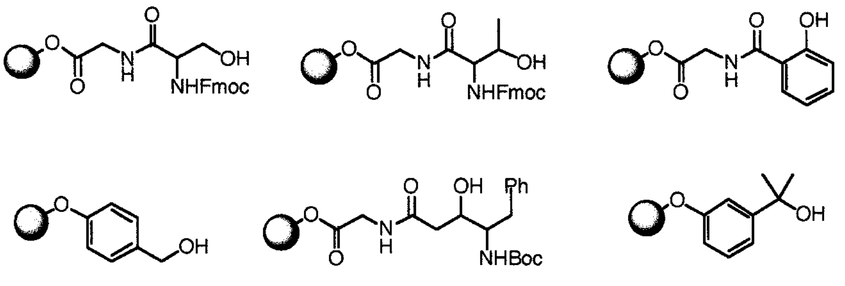


Figure 1. (a) Positive test (microscope, 10×) of TCT–AliR (red) together with negative beads (uncoloured); (b) photograph of test tubes containing from the left: negative beads, yellow (TCT–fluorescein) beads and red (TCT–AliR) beads; (c) Image taken from the fluorescence microscope (20×) of a bead treated with TCT–fluorescein having 1% of free OH

On the other hand, a sample of the Wang-Gly-Fmoc resin, treated under the same conditions and washed several times with DMF to give a clear solution, resulted as gel-type uncoloured beads (Fig. 1a). To better appreciate the variation of colour we suggest a comparison should be made with definitely negative beads (swelled), previously submitted to the entire test.

To check the potential of our method we tested several compounds linked to a resin and containing an OH group and always obtained positive results (see Scheme 3). All the products employed have primary, secondary and tertiary OH and were linked either to Wang or trityl resins and their identity was confirmed by FT-IR spectra[12] or by the analysis of the products after cleavage (when it was possible). It is noteworthy to see that the TCT–AliR method can be employed to monitor the loading of the first molecule on a Wang type resin.



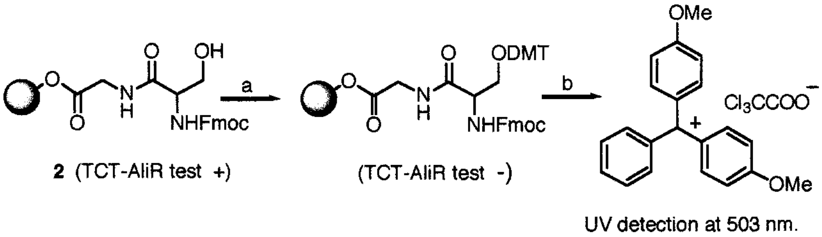
Scheme 3. Molecules tested

To verify the sensitivity of the method we prepared a mixture of *N*-Fmoc-Ser-OH and *N*-Boc-Ala-OH in a 5:95 ratio and coupled this mixture on a Wang resin loaded with Gly-NH_2. The coupling was monitored using the Kaiser test and the amount of Fmoc determined by UV quantitation of the dibenzylfulvene adduct at 301 nm.[1d] The presence of Fmoc groups on the resin (which was related to Ser and consequently to the presence of OH) was determined to be 4%

of the theoretical loading. This resin was submitted to a TCT–Ali-R test and gave a positive pale orange colour if compared with the test carried out on a negative resin. The fluorescein as the dye and submitting the resin to a fluorescence microscope. Fig. 1c shows an image taken at the fluorescence microscope of a resin having 1% of free OH.

As the first step of the test is a nucleophilic substitution on TCT, other nucleophiles, such as primary and secondary amines (and thiols), give a positive test. A COOH group is incompatible as TCT may induce the lactonisation of hydroxy acids. Finally, the test cannot be used, for example, to control the transformation of an OH group into a tosylate, as AliR may give nucleophilic substitution on a tosylate and colours the beads in red.

An indirect quantitative determination of the amount of primary and secondary OH present on the resin can be performed with our TCT–AliR test (Scheme 4).



Scheme 4. (a) DMT–Cl, pyridine, 4 h, rt; (b) CCl_3COOH, CH_2Cl_2

Resin **2** was coupled with dimethoxytrityl chloride (DMT-Cl, pyridine as solvent, 3 h) until the TCT–AliR test was negative. Then the resin was dried and from an aliquot of the resin (~5 mg) the dimethoxytrityl group was removed using 3% trichloroacetic acid in dichloromethane. The resulting orange solution, which was formed by the released dimethoxytrityl cation, was measured on a spectrophotometer at 503 nm.[13] The loading values obtained were in good accord (±3%) with the corresponding values determined by Fmoc quantitation. It is noteworthy that the DMT loading on the OH group is a time consuming experiment that may be carried out if the amount of free OH is required. Moreover, without a colourimetric test for the free OH, it is impossible to evaluate the complete loading of DMT on the resin and the test becomes very uncertain.

In conclusion, we have developed a new simple method to visually detect the presence (or the absence) on an OH group on the resin. The method uses commercially available inexpensive reagents and may also be used to load different dyes or fluorescent molecules on the resin for additional surface investigations.

Acknowledgements

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