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Malachite green, a valuable reagent to monitor the presence of free COOH on the solid-phase

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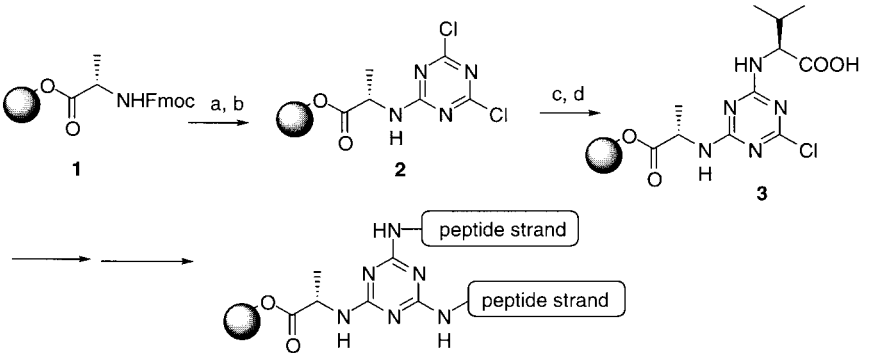
Abstract

A rapid and sensitive colour test to monitor the presence of a COOH in a polystyrene resin was developed. The method could also be used for a quantitative determination.

Keywords: solid-phase synthesis; dyes; carboxylic acids; colourimetric assay.

Following our interest in application of the combinatorial approach to organic chemistry,[1] we were interested in preparing a library of peptidic strands linked around a triazine template (Scheme 1). Thus, after the attachment of a first amino acid (Ala) to a Wang type resin and further deprotection, we loaded a dichloro-triazine moiety obtaining compound **2**. The formation of this compound was easily monitored with the Kaiser test[2] that showed that all the NH_2 present on the resin were reacted.



Scheme 1. (a) Piperidine, DMF, 30 min; (b) TCT, NMM, THF, 2 h, rt; (c) H-Val-OH, TMSCl, Et_3N, 12 h rt; (d) MeOH

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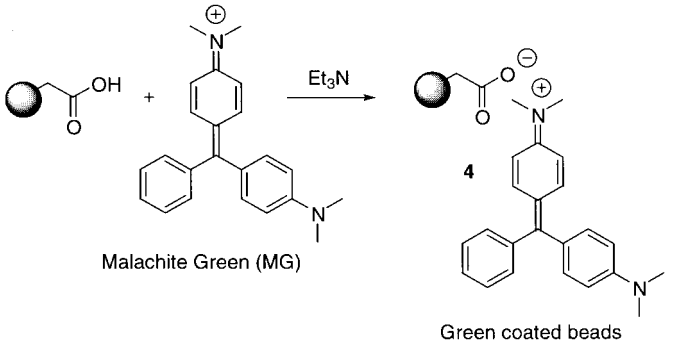
Then we tried to load a free amino acid on the first chlorine of the triazine. The amino acid was dissolved in DMF using trimethylchlorosilane and triethylamine,[3] the solution added to the resin and the mixture stirred (bubbling nitrogen) for 12 h. At this point we realised that we had no chance of rapidly monitoring the formation of product **3** in Scheme 1. We must remove some beads from the solution, rinse them several times, dry them very well and analyse them by FT-IR analysis. As the process was time consuming (the resin must be dried for at least 12 h to obtain a good spectrum) we looked for a spot test for monitoring free carboxylic acids on resins. We found that the bromophenol blue test for amines could be used for carboxylic acids,[4] as an acidic surface may induce a colour change in the indicator solution. Unfortunately, in our case, the presence of basic centres together with the COOH gave unreliable results.

We thought that an amine containing dye could be used to monitor the presence of a COOH with the formation of a coloured salt on the beads. We tried several basic dyes and stains and we found that malachite green (MG) (Scheme 2)[5] was an effective indicator of the presence of a (carboxylic) acid on polystyrene resins. MG, sold as the oxalate salt, is positively charged and reacts with a carboxylate turning the beads green.



Scheme 2.

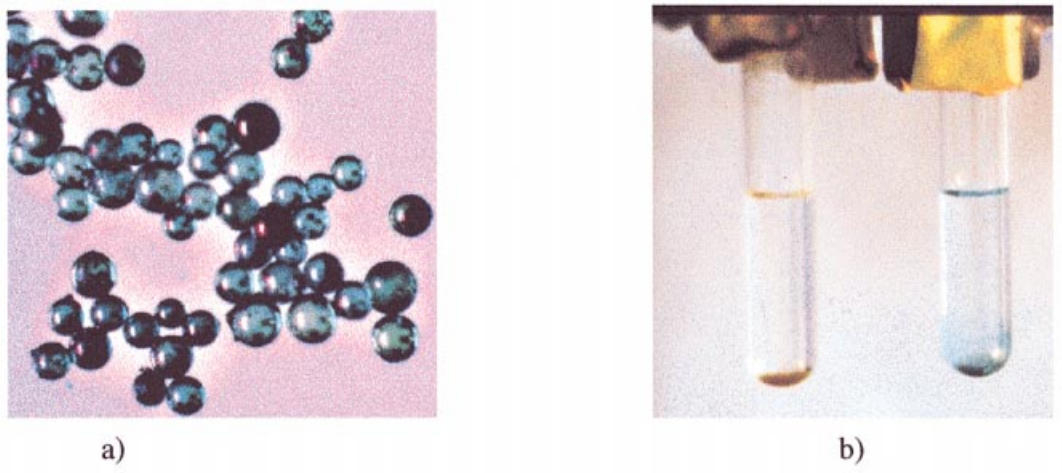
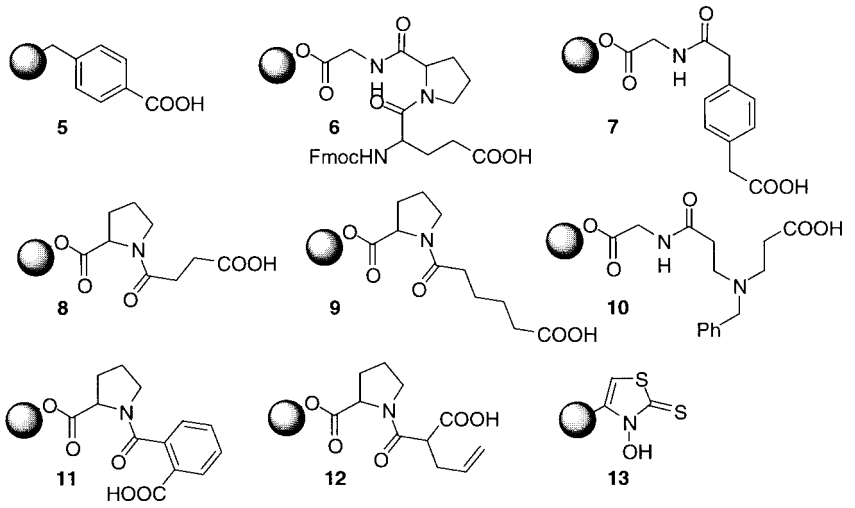


Figure 1. (a) Positive test (microscope, 10×) of COOH containing beads treated with MG.; (b) photograph of test tubes containing, from the left: negative beads, and green (MG positive) beads

The test procedure is very simple: A few beads of the resin are washed several times with the solvent employed in the reaction that we want to monitor, followed by MeOH. Afterwards 1 mL of a 0.25% solution of MG–oxalate in EtOH is added followed by a single drop of pure Et_3N. After 2 min at room temperature the green solution is discarded and the beads rinsed several times with EtOH (or MeOH) until the solution remains clear. In the presence of COOH the beads are coloured dark green, alternatively they appears as clear gel pearls. The colour of the beads can be visualised with the microscope (10 times magnification is enough, see Fig. 1a) or even with the naked eye (Fig. 1b).

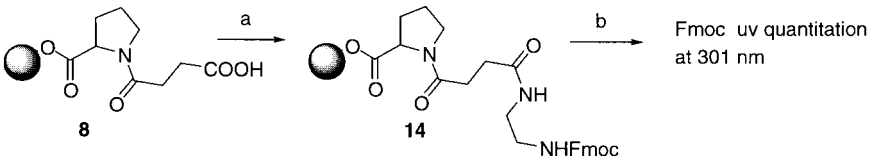
We applied this test to several carboxylic acids loaded on polystyrene resins (see Scheme 3) always obtaining excellent results. It is noteworthy to see that the test may be used to evaluate the selective deprotection of the glutamic acid side chain (**6**),[6] and the mono-functionalisation of a dicarboxylic acid with a resin linked amine (**7**–**10**).[7] Also, aromatic (**11**) and substituted carboxylic acids (**12**) or hydroxamic derivatives (**13**) can be easily detected. The test is also very sensitive (1% of COOH on the resin can be detected) as the green colour is easily appraised by eye.



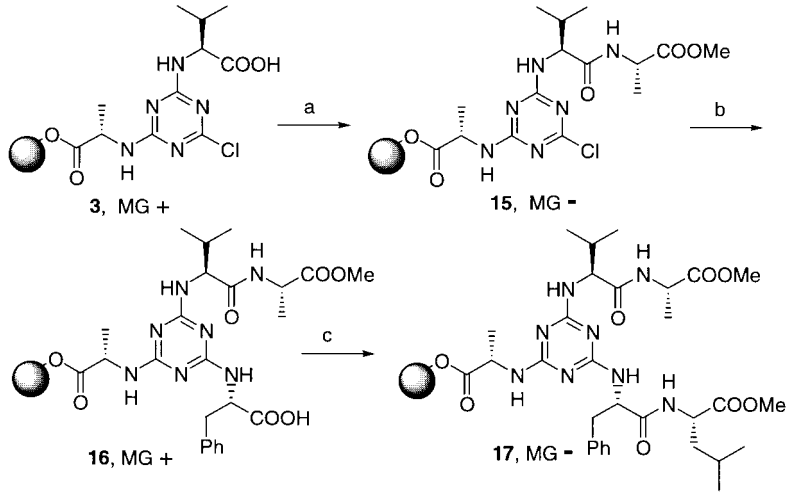
Scheme 3.

We also tried to perform a direct quantitative test monitoring the amount of MG released from the green beads when treated with a DMF solution containing oxalic acid. Unfortunately, the resulting DMF solution (very diluted in MG) was unstable to the light and we were not able to obtain reproducible UV spectra. We found it more practical to perform a coupling of the acid with *N*-Fmoc 1,2-diaminoethane using a classical coupling agent (PyBOP, DMTMM or others), monitoring the efficiency of the coupling with MG (negative test means all the COOH reacted), and finally determining the amount of the Fmoc present by deprotection and quantitative determination of the piperidine-dibenzylfulvene adduct at 301 nm (Scheme 4).

Thus, with our new colour test, we could rapidly accomplish the preparation of a library of products as **17** (Scheme 5). Compound **3** was reacted with H-Ala-OMe in the presence of PyBOP and the formation of **15** was indicated with a negative MG test. Afterwards, reaction with H-Phe-OH in DMF at 60°C for 24 h in the presence of TMSCl and Et_3N followed by MeOH gave **16** (MG-test positive) that was coupled with H-Leu-OMe (PyBOP, DIPEA, DMF)



Scheme 4. (a) $FmocHNCH_2CH_2NH_2$, PyBOP, DIPEA, DMF, 3 h, rt; (b) piperidine, DMF, CH_2Cl_2



Scheme 5. (a) H-Ala-OMe, PyBOP, DIPEA, DMF, 3 h, rt; (b) H-Phe-OH, TMSCl, Et_3N, DMF, 24 h, 60°C; (c) H-Leu-OMe, PyBOP, DIPEA, DMF, 5 h, rt

to give **17** (MG-test negative). The formation of the product was confirmed by spectroscopic analysis after the cleavage with TFA:CH_2Cl_2, 1:1.

In conclusion, we have developed a rapid, sensitive colour test that uses commercially available reagents to monitor the presence (and eventually the amount) of COOH on a polystyrene resin.

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5. Abbreviations used through the paper: MG = malachite green; TCT = 2,4,6, trichloro-[1,3,5]-triazine; TMSCl = chlorotrimethylsilane; NMM = *N*-methyl morpholine; PyBOP = (benzotriazol-1-yloxy)tripyrrolidino-phosphonium hexafluorophosphate; DIPEA = diisopropyl ethyl amine.
6. Compound **6** was obtained by deprotection of the allyl ester with $Pd(PPh_3)_4$ and dimedone in DMF.
7. Compounds **7**–**10** were obtained by coupling a large excess of the acid and the resin linked amine.