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Fast and easy detection of aromatic amines on solid support

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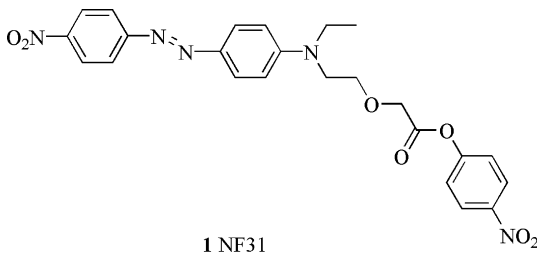
Abstract—The use of *p*-nitrophenyl ester **1** has been shown to offer a reliable method for the detection of free aromatic amines. As little as 3.4 μmol g^{-1} of free aniline amino groups can be detected. The method has shown to be more sensitive for the detection of sterically hindered aromatic amines than the existing alternative based on reaction with chloranil.

1. Introduction

The success of solid phase peptide synthesis (SPPS) has been largely influenced by the development of colorimetric methods for the detection of free amines on solid support, allowing fast and reliable evaluation of the amino acid coupling efficiency. In SPPS, a variety of colorimetric methods is available.[1] Our group made a contribution in this area with the development of *p*-nitrophenylester **1**, which allows reliable monitoring of coupling reactions with even very sterically hindered primary and secondary amines (see Fig. 1).[2]

With the event of combinatorial chemistry, other types of chemistry are also performed more and more on solid supports, increasing the need for sensitive tests to monitor the completeness of specific reactions on a solid support.



1 NF31

Figure 1. Structure of *p*-nitrophenylester **1**.

We recently became interested in the acylation of anilines on solid support. In contrast to the plethora of tests available to detect free aliphatic amines on solid support, few methods are available for monitoring reactions with aromatic amines. The two most commonly used color tests, Kaiser and TNBS, are not applicable in case of aromatic amines. To date, only the so-called chloranil test has been reported to monitor the progress of these reactions with high sensitivity.[3] The use of malachite green isothiocyanate (MGI) is also being described for the detection of resin bound anilines but the authors do not discuss the sensitivity of the test.[4] Other drawbacks of the MGI method are the need for relatively large quantities of resin (4 mg) and a long reaction time (1 h at ambient temperature). In addition, recently the use of 2-amino-3-chloro-5-nitro-1,4-naphtoquinone was reported as a general method for the detection of resin bound amines. However, whereas reaction with aliphatic amines occurs at room temperature, detection of aromatic amines requires heating under microwave irradiation at 80 °C and no data on sensitivity are given in this case.[5]

Here we report on the use of **1** as an alternative and complementary way of detecting free aromatic amines on solid support. Because aromatic amines are frequently used as building blocks in medicinal chemistry and are readily accessible via reduction of a nitro group,[6] the current method could have widespread use and allow the monitoring of aromatic amine acylation with a greater sensitivity.

2. Results and discussion

p-Nitrophenylester **1** is easily prepared starting from commercially available and cheap Disperse Red 1 via

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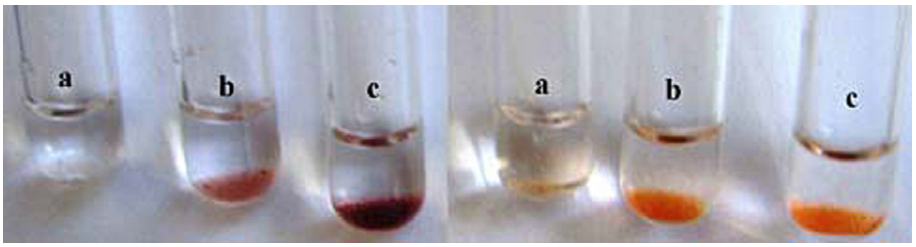


Figure 2. Detection of resin bound *p*-Abz with chloranil (left) and NF31 (right). The amount of free amine is (**a**) 3.4 μmol g^{-1}; (**b**) 13 μmol g^{-1}; (**c**) 18 μmol g^{-1}.

a high yielding three-step procedure.[2a] Furthermore, shortly after the description of its use in the monitoring of peptide coupling reactions, it was commercialized. However, the reaction with aromatic amines has not been described before. We have now observed that upon incubation of resin beads with free aromatic amines in an acetonitrile solution of **1**, also in this case intensely red colored beads are produced. In order to tackle the sensitivity issue, mixtures of FmocGlyOH and Boc-GlyOH were coupled in varying ratios to Tentagel–S–NH_2 (loading 0.21 mmol/g) using the PyBOP/DIPEA protocol. After selective Fmoc-deprotection with 20% piperidine in DMF, Fmoc-protected *p*-aminobenzoic acid (Fmoc–*p*-Abz) was coupled, again using PyBOP and DIPEA. The amount of Fmoc–*p*-Abz on the resin was determined by measuring the absorbance of the piperidine–fulvene adduct at 301 nm, giving values of 3.4, 13, and 18 μmol g^{-1}. Finally, the Fmoc-group was deprotected with a 20% piperidine/DMF solution.

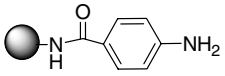
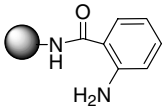
In order to check if the sensitivity of the colorimetric tests is influenced by sterical hindrance we further prepared resin loaded with 5.4 and 11 μmol g^{-1} of *o*-Abz in the same manner.

The resins were treated with a 0.002 M solution of **1** in acetonitrile and heated at 70 °C for 10 min. For comparison, fractions of these resins were also exposed to the chloranil test.[3] Figure 2 compares the results of both tests.

As can be observed from the color intensity, the NF31 test is slightly more sensitive than the chloranil test for the detection of *p*-Abz at low substitution levels. Whereas in the case of the chloranil test, resin **a** appears almost white, coloration with NF31 is clearly visible (see Table 1).

We repeated the test for the *o*-Abz and as noticed by Lam et al., treatment with chloranil gave a weaker intensity than in the case of *p*-Abz.[3] We were pleased to find that NF31 gave a clearly visible coloration of the beads (see Fig. 3).

Table 1. Detection of resin bound aromatic amines with NF31

	Name	Resin substitution (μmol g^{-1})	Result
	**a**	3.4 (~2%)	+
	b	13 (~5%)	++
	c	18 (~9%)	++
	**d**	5.4 (~3%)	+
	e	11 (~5%)	++

Tentagel–S–NH_2 resin (loading 0.21 mmol/g) was used in these experiments.

In conclusion, reagent **1** offers a simple and sensitive test for the monitoring of coupling reactions to free aromatic amines during solid phase synthesis. Previously validated for the monitoring of peptide coupling, the use of NF31 is now extended to the detection of free aromatic amines, rendering it a versatile and useful tool for solid phase chemistry.

3. General remarks

The coupling reactions were performed in peptide grade DMF purchased from Biosolve. Tentagel–S–NH_2 resin was purchased from Rapp Polymers. Amino acids and PyBOP were purchased from Novabiochem. Piperidine (99%) and DIPEA (99.5%) were obtained from Aldrich. All reagents and solvents were used as received without further purification. *p*-Nitrophenylester **1** was synthesized as previously described.[2] The UV-measurements were carried out with a VARIAN-CARY 3E spectrophotometer. Color tests were performed in heavy-walled test tubes of ca. 35 × 6 mm.

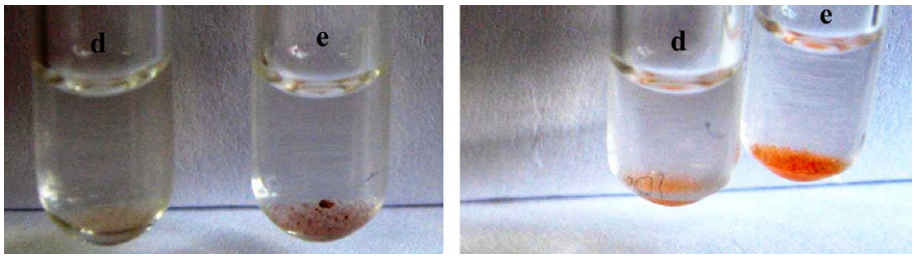


Figure 3. Detection of resin bound *o*-Abz with chloranil (left) and NF31 (right). The amount of free amine is (**d**) 5.4 μmol g^{-1}; (**e**) 11 μmol g^{-1}.

3.1. General Fmoc-deprotection

A 20% piperidine/DMF solution (10 mL/g resin) is added to the beads and the resin is shaken for 1 min. The solution is drained and the resin is washed with DMF/MeOH/DCM. This protocol is repeated twice with shaking, respectively, for 5 and 10 min.

3.2. Loading determination

A portion of the resin (10–20 mg) is transferred to a 10 mL flask and a 20% piperidine/DMF solution is added. After 20 min, the suspension is shaken, the resin is left to settle and the liquid is transferred to a cuvette. The absorbance of the piperidine–fulvene adduct is measured at 301 nm and this value is used to calculate the loading via a calibration curve.

3.3. Preparation of resin a

A stock solution of FmocGlyOH is prepared by dissolving FmocGlyOH (30 mg, 100 μmol) in DMF (1 mL). Then 20 μL of the stock solution is added to a solution of BocGlyOH (14 mg, 82 μmol) in DMF (980 μL). The coupling reagent PyBOP (44 mg, 84 μmol) and DIEA (29 μL, 168 μmol) are added after which the resulting mixture is added to the pre-swollen Tentagel–S–NH_2 (100 mg, 21 μmol). The suspension is shaken for 2 h and the completion of the reaction is checked via the TNBS and NF31 test. The resin is Fmoc deprotected. A 0.1 M stock solution of Fmoc–*p*-Abz (216 mg, 600 μmol) in DMF (6 mL) is prepared. To the resin (50 mg) is then added 1 mL of the Fmoc–*p*-Abz stock solution and the resin is shaken for 3 h. The NF31 test was negative, indicating a complete coupling. The loading was 3.4 μmol g^{-1}. Finally, the resin was Fmoc deprotected.

3.4. Preparation of resins b and c

By using the same stock solution of FmocGlyOH but by changing the added volume, different substitution levels were obtained. For the coupling of Fmoc–*p*-Abz the same 0.1 M stock solution is used. The loading was 13 μmol g^{-1} for **b** and 18 μmol g^{-1} for **c**.

3.5. Preparation of resins d and e

A similar strategy as in the case of *p*-Abz was followed. The loading was 5.4 μmol g^{-1} for **d** and 11 μmol g^{-1} for **e**.

3.6. Chloranil test

As previously described by Lam et al., a yellow 2% chloranil/DMF solution is prepared.[3] The desired resin (~1 mg) is placed in a test tube and 100 μL of the chloranil solution is added. The suspension is heated for 5 min at 100 °C. Because of the yellow background color we decided to wash the resin with DMF/MeOH/DCM. At the end, the beads were shrunk in MeOH and a brown to purple color was observed (depending on the amount of free aromatic amines present).

3.7. NF31 Test

A 0.002 M solution of **1** in CH_3CN is prepared to give a red solution. The desired resin (~1 mg) is placed in a test tube and 50 μL of the NF31 solution is added after which the suspension is heated for 10 min at 70 °C. Then the beads are washed with DMF/MeOH/DCM and shrunk with MeOH to give a pink to red color (depending on the amount of free aromatic amines present).

Acknowledgments

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