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A new analytical method for anchoring quantification of amines on resin support

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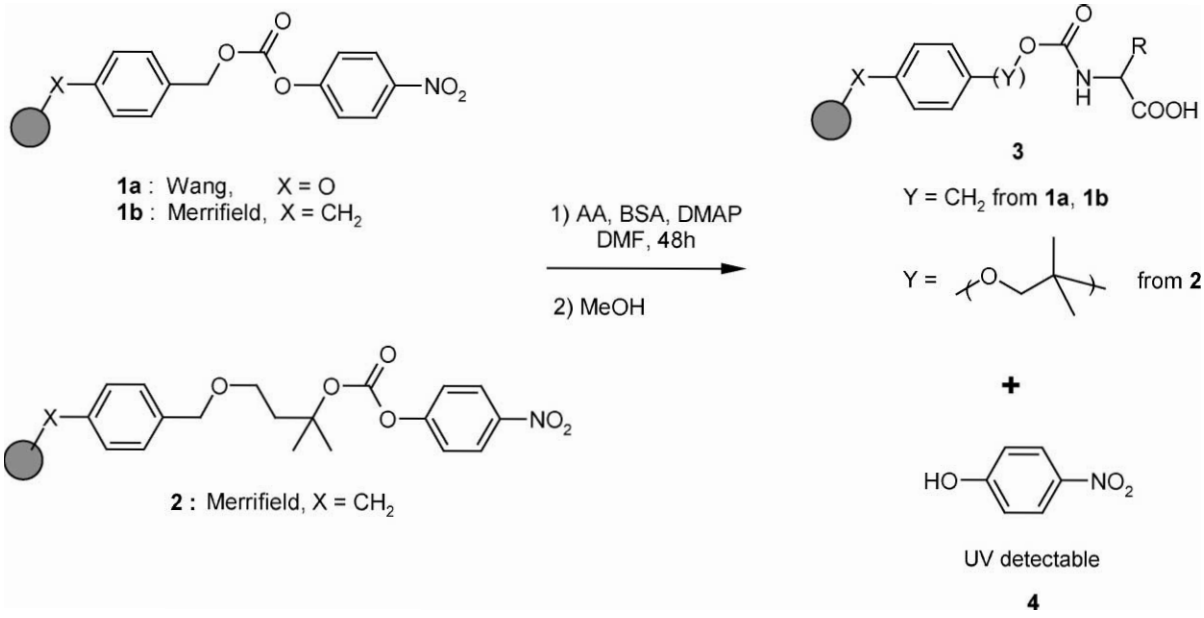
Abstract—A rapid and quantitative method for monitoring the efficiency of coupling of amino compounds to polystyrene resin through a carbamate linker has been developed. *para*-Nitrophenyl carbonate activating group has been shown to release a valuable chromophore for quantitatively monitoring the progress and the yield of the reaction.

The combinatorial synthesis of a large collection of compounds has rapidly emerged as a powerful method for identifying biologically active molecules.[1–3]

Significant advances have been made in the rapid synthesis of peptide and oligonucleotide mixtures and more recently in the synthesis of small organic non-peptide libraries on solid support.[4] In addition, a significant number of organic reactions have been studied on solid phase, and a variety of new chemistry developed.[5,6] In most cases cleavage of the product from support and full characterisation of the target compound have been



Scheme 1.

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accomplished to evaluate individual reactions. However, the search for methods of monitoring the efficiency of anchoring before undertaking reactions on support, when mixtures of compounds are generated or multi-step sequences are required, creates a demand for new and selective quantitative tests.

In the course of our work on non-peptide chemistry we found the need for coupling amino acids to polystyrene resin via their N-terminus moiety. In addition we were interested in developing a rapid and quantitative method for monitoring the efficiency of the coupling of our amino acids, before undertaking any further elaboration. Being the carbamate moiety a suitable linker for our purposes we decided to use the activated *para*-nitrophenyl carbonate resins **1a**, **1b** and **2** as supports. *para*-Nitrophenylcarbonate activating group has been previously reported for immobilizing amines,[7–13] or aminidines[14] as well as for coupling of amino acids.[15] Beside its efficiency in promoting the anchoring of amino moieties, the activated *para*-nitrophenyl carbonate resins, releasing *para*-nitrophenol in solution, would also afford a valuable chromophore for quantitatively monitoring the efficiency of the coupling (Scheme 1). The method we developed is indeed based on the determination of the amount of *para*-nitrophenolate generated under basic conditions (yellow chromophore at pH >7.6, $\lambda_{max}=434$ nm) once released during the coupling reaction. The reported method has been set up for both loading estimation of resins **1a**, **1b**, **2** and calculation of the yield of the amino acid coupling reaction. Definitely, these two values could be easily determined through a simple UV measurement according to the equation (dil = dilution as mL, wt = g of resin).

$$\textit{Substitution}\ (\text{mmol/g}) = A_{434} \times V \times \text{dil}/(\varepsilon_{434} \cdot \text{wt})$$

Obviously, for quantitative analysis we had to find the extinction coefficient (ε) of a basic solution of **4** which was determined as 32,800 M^{-1} cm^{-1}, at $\lambda_{max}=434$ nm.

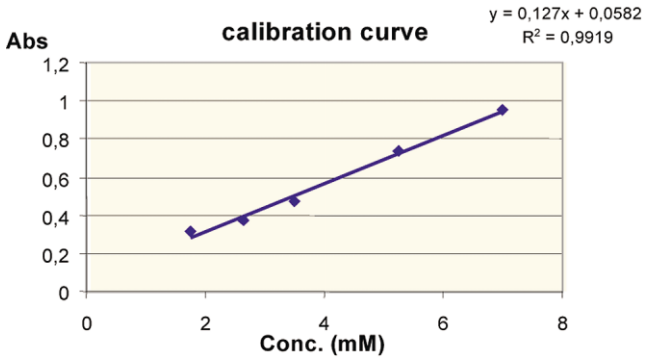


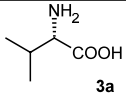
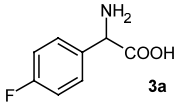
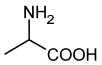
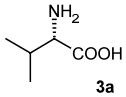
Figure 1. UV calibration curve of chromophore **4**.

The calibration curve was also established using five different concentrations of chromophore solutions, a good correlation factor $R^2=0.9919$ was found, as reported in Figure 1.

Polystyrene Wang resin (loading 0.84 mmol/g) was thus reacted with *para*-nitrophenylchloroformate[15] to obtain activated resin **1a** with high yield (Scheme 1). The efficiency of this reaction was proved upon *para*-nitrophenol cleavage: a known amount (~20 mg, re-calculated loading 0.72 mmol/g) of resin **1a** was reacted with 1 mL of a 20% solution of piperidine in DMF for 1 h. After filtration 20 μL of the obtained solution was diluted to 25 mL with DMF to obtain a $\sim 2\times10^{-5}$ M concentration, suitable for UV measurement. The absorbance of the sample, measured against the reagents blank at 434 nm, showed a convertion of 90% (loading 0.65 mmol/g).

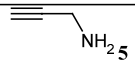
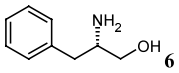
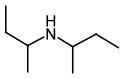
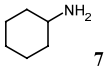
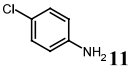
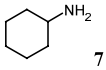
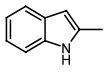
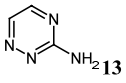
The same method was applied to calculate the efficiency of activation of a Merrifield resin **1b** (measured *para*-nitrophenol loading 1.30 mmol/g over 2.0 mmol/g of initial loading) and of a different *para*-nitrophenylcarbonate activated resin **2**, which was prepared as reported.[16] In the latter case a lower value of the

Table 1. Loading of different aminoacids on activated resin **1a** and **2**

Entry	AA	Loading (mmol/g) of the resin [a,b)]	Yield %	Loading (mmol/g)of the AA
1	 **3a**	0.65 [a)]	88	0.57
2	 **3a**	0.65 [a)]	>95	0.63
	D,L **3b**			
3		0.65 [a)]	90	0.59
	D,L **3c**			
4	 **3a**	0.44 [b)]	95	0.39

a) Activated resin **1a**
b) Activated resin **2**

Table 2. Loading of different amines on activated resin **1a** (0.65 mmol/g) or **1b** (1.34 mmol/g)

Entry	AA	Yield %	Loading	Entry	AA	Yield %	Loading
1	 **5**	>95	0.65 [a)]	6	 **9**	75	1.06 [c)]
2	 **6**	78	0.5 [a)]	7	 **10**	58	0.78 [c)]
3	 **7**	89	0.6 [a),b)]	8	 **11**	62	0.84 [c)]
4	 **7**	90	1.27 [c)]	9	 **12**	31	0.41 [c)]
5	 **8**	80	1.15 [c)]	10	 **13**	87	1.22 [c),a)]

a) Loaded on resin **1a**
b) In this case coupling efficiency was also confirmed by determining the amine salt recovered upon TFA cleavage
c) Loaded on resin **1b**

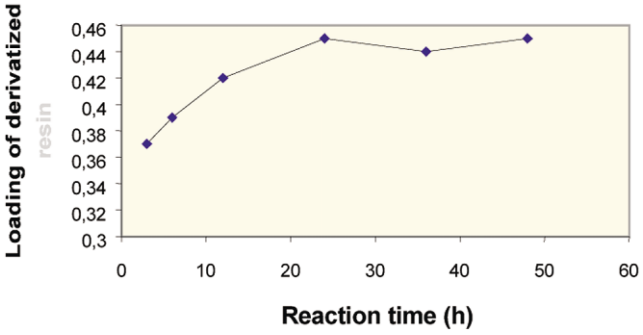


Figure 2. Monitoring of the coupling reaction of **5** on activated resin **1a**.

loading was recovered (measured 0.44 mmol/g over 1.0 mmol/g) probably because a small fraction of the less reactive tertiary alcohol groups remained free. This fact has already been observed in similar reactions,[17] and we believe that the possibility of knowing the exact loading of amines on this support can be a useful improvement for the further development of this *t*-Boc like linker.

The yield of amino acids coupling was then determined. In a typical experiment a slurry of a known amount (~500 mg) of resin **1a** in 3 mL of dry DMF was treated with a DMF solution (3 mL) prepared with 4 equiv. of AA, 1 mL of BSA and 100 mg of DMAP.[15] The yield of coupling was then monitored by taking a sample of 10 μL, adding 20 μL of a 20% of piperidine in DMF and diluting to 25 mL with DMF. The absorbance measurements were generally repeated for three times. The obtained results are reported in Table 1.

Three different amino acids were examined and in all cases high yields of coupling were observed, even when resin **2** was used (Table 1, entry 4). The analyses were also repeated using lower amounts of resin in the coupling reaction and comparable results were obtained working with a 50 mg scale.

In order to exemplify its generality and efficiency the method was extended to several amines. The results obtained are reported in Table 2. A series of primary amines was used first, and in all cases a good yield of coupling was recovered, even when hindered substrates like *t*-butylamine (entry 5) or phenylalaninole (entry 2) were used. To confirm the accuracy of the method less reactive amines were tested. Secondary cyclohexylamine showed a comparable reactivity (entry 6), but when more hindered *sec*-isobutylamine was used (entry 7) a lower yield was measured as expected. This trend was confirmed also in the case of aromatic amines (entries 8 and 9), in particular a very low yield was observed in the coupling of α-methylindole and this low value was not increased after repeating the reaction three times. It is known that protection of the indole nitrogen can be troublesome[18] and in our case this fact was probably enhanced by the hindrance due to the methyl group in the α-position. Loading of aminotriazine (Table 2, entry 10) on activated Merrifield resin **1b** was also

determined. Also in this case we observed a high yield of coupling which can be explained by the lower aromaticity of this heterocycle.

As a final remark using this procedure, monitoring of the reaction progress was also possible as shown in Figure 2.

Loading of propargylamine **5** on resin **1** was in fact measured at different times showing that the reaction was almost complete after 24 h. This was also confirmed by FTIR analysis by checking the increase of the strong absorption of the ≡C–H stretching at 3290 cm^{-1}.

Rapid determination of both percent of conversion and time required for reaction completion, avoiding tedious cleavage of intermediates at each checkpoint, can be achieved. Knowledge of the time required for a reaction step to reach completion is crucial during the optimisation phase as well as during library synthesis on solid-phase, therefore this methodology is indeed very useful when applicable to kinetic studies.

In conclusion, we have developed a rapid, sensitive and quantitative test for monitoring the yield of coupling of amino compounds on polystyrene resins through a carbamate linker. The method can be efficiently used for loading estimation of the derivatised resin as well as for reaction kinetic studies.

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References

1. Gallop, M. A.; Barrett, R. W.; Dower, W. J.; Fodor, S. P. A.; Gordon, E. M. *J. Med. Chem.* **1994**, *37*, 1233.
2. Gordon, E. M.; Barrett, R. W.; Dower, W. J.; Fodor, S. P. A.; Gallop, M. A. *J. Med. Chem.* **1994**, *37*, 1385.
3. Hermkens, P. H. H.; Hamersma, H. *J. Comb. Chem.* **1999**, 307.
4. Dolle, R. E. *J. Comb. Chem.* **2002**, 369.
5. Nefzi, A.; Ostresh, J. M.; Houghten, R. A. *Chem. Rev.* **1997**, *97*, 449.
6. Sammelson, R. E.; Kurth, M. J. *Chem. Rev.* **2001**, *101*, 137.
7. Dixit, D. M.; Leznoff, C. C. *J. Chem. Soc., Chem. Commun.* **1977**, 798.
8. Dixit, D. M.; Leznoff, C. C. *Isr. J. Chem.* **1978**, *17*, 248.
9. Rajii, B.; Kogan, T. P. *Tetrahedron Lett.* **1997**, *38*, 3373.
10. Veerman, J. J. N.; Ritjes, F. P. J.; vanMaarseveen, J. H.; Hiemstra, H. *Tetrahedron Lett.* **1999**, *40*, 6079.
11. Huang, W.; Scarborough, R. M. *Tetrahedron Lett.* **1999**, *40*, 2665.
12. Hall, S. E. *Mol. Diver.* **1999**, *4*, 131 and references cited therein.
13. Li, W.-R.; Lin, Y.-S.; Yo, Y.-C. *Tetrahedron Lett.* **2000**, *41*, 6619.
14. Roussel, P.; Bradley, M. *Tetrahedron Lett.* **1997**, *38*, 4861.
15. Dressman, B. A.; Spangle, L. A.; Kalder, S. W. *Tetrahedron Lett.* **1996**, 937.
16. Leger, R.; Yen, R.; She, M. W.; Lee, V. J.; Hecker, S. J. *Tetrahedron Lett.* **1998**, *39*, 4171.
17. Hernandez, A. S.; Hodges, J. C. *J. Org. Chem.* **1997**, *62*, 3153.
18. Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis*; John Wiley & Sons: New York, 1999.