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Alcohols immobilization onto 2-chlorotritylchloride resin under microwave irradiation

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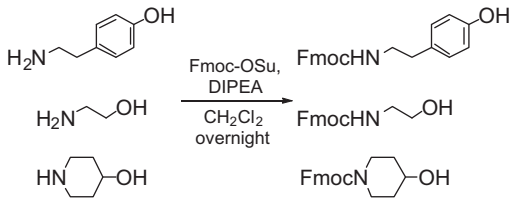
ABSTRACT

The immobilization of alcohols onto 2-chlorotritylchloride resin using microwave irradiation was studied. Three different Fmoc-aminoalcohols were tested: the phenol-like Fmoc-tyramine, the primary alcohol Fmoc-ethanolamine, and the secondary alcohol Fmoc-4-hydroxypiperidine. Several reaction conditions were evaluated: different bases, reaction times, temperatures, and concentrations. Microwave immobilization resulted is effective in binding to the resin all three types of alcohols with loadings which were superior or comparable to the 'classical' methods in shorter time and without employing toxic and racemizing reagents. This method resulted also useful for the immobilization, through the hydroxyl group, of FmocTyrOAll, FmocSerOAll, and FmocThrOAll, important building blocks for the synthesis of cyclic peptides.

2-Chlorotrityl chloride resin (2CTC) is widely employed in solid phase organic and peptide synthesis, because it overcomes several problems related to the use of other types of resins, such as Merrifield and Wang resins.[1] For example, unlike Wang resin, attachment of α-amino acids to 2CTC is free from enantiomerization and other side reactions, such as diketopiperazine formation. Moreover, extremely mild acidolysis conditions make possible the cleavage of protected peptide segments from the resin.[2,3] However, the greater advantage of 2CTC is its vast versatility since almost any nucleophilic functional group is able to link to this resin: in fact, besides carboxylic acids, trityl resins are utilized, under mild conditions, to bond amines, thiols, guanidines, imidazoles, and other basic heterocycles.[4–8]

Trityl chloride resins are also employed for the immobilization of alcohols[9,10] but in this case the attachment to the solid support is not as easy as for other functional groups: often the use of strong nucleophiles, such as DMAP, or toxic bases, such as pyridine, dry solvents, and long reaction times (from 2 to 96 h) are required.[10–19] In addition, the reaction yields are usually very low,[20] reaching, in the best cases, a resin loading of 0.2–0.3 mmol/g. Since microwave (MW) irradiation has been successfully employed for more than 20 years in organic synthesis, in order to obtain faster reaction times and/or higher yields respect to conventional heating conditions,[21–23] we decided to apply this technique to the immobilization of alcohols onto 2CTC. Thus, in this Letter we study the effects of MW irradiation on the attachment of primary, secondary, and phenol-like alcohols to the 2CTC. As model compounds for this study we chose tyramine, ethanolamine, and 4-hydroxypiperidine, phenol-like, primary and secondary alcohols, respectively: the amine moiety of these molecules was protected as Fmoc derivative,[24] (Scheme 1) being one of the most frequently used amine-protecting group in the solid phase synthesis. The cleavage of the Fmoc group after the reaction between 2CTC and the chosen alcohols allowed us to verify the resin loading by UV analysis.[25] Using a polystyrene 2CTC loading of 1.55 mmol/g, a series of experiments were carried out in order to test the effects of different bases, reaction times, concentrations, and temperatures in MW assisted loading of Fmoc-amino alcohols onto 2CTC.[26]

With the aim of finding less toxic bases than pyridine and DMAP, often used in the immobilization of alcohols onto 2CTC, we tested the MW reaction, maintaining constant temperature (50 °C), time (30 min), and concentration (80 mM), with the presence of different tertiary bases: diisopropylethylamine (DIPEA), triethylamine (TEA), *N*-methylmorpholine (NMM), and 1,5-diazabicyclo[4.3.0]non-5-ene (DBU); using these type of bases the formation of activated species was avoided, so the reaction



Scheme 1. Synthesis of Fmoc-aminoalcohols.

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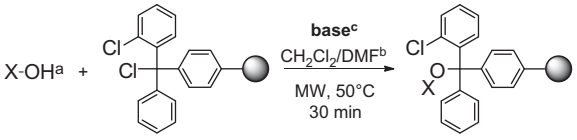
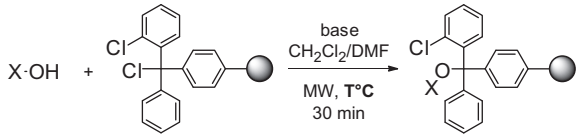
Table 1
Effect of the bases in microwave-assisted loading of Fmoc-amino alcohols onto 2-chlorotrityl resin



Base	Loading (mmol/g)		
	FmocHN–OH	FmocHN–OH	FmocN–OH
DIPEA	0.380	0.132	n.r.d.[g]
TEA	0.388	0.204	n.r.d.[g]
NMM	0.246	0.304	n.r.d.[g]
DBU[d]	0.320	0.560	0.200
Pyridine	n.r.d.[g]	0.236	n.r.d.[g]
DIPEA, DMAP[e]	0.120	0.052	n.r.d.[g]
DIPEA, DMAP[f]	0.064	0.070	n.r.d.[g]

[a] 2 equiv, [b] 1.8 ml CH_2Cl_2 and 0.1 ml DMF for 50 mg of resin, [c] 8 equiv.
[d] Loading calculated after coupling with FmocGlyOH.
[e] 0.2 equiv.
[f] 1 equiv.
[g] No reaction detected.

Table 2
Effect of the temperature in microwave-assisted loading of Fmoc-amino alcohols onto the 2-chlorotrytyl resin



Temperature (°C)	Loading (mmol/g)		
	FmocHN–OH	FmocHN–OH	FmocN–OH
50	0.380[a]	0.304[b] 0.560[c]	0.200[c]
100	0.386[a]	0.315[b] 0.565[c]	0.208[c]

[a] DIPEA.
[b] NMM.
[c] DBU (loading calculated after coupling with FmocGlyOH).

would be free from epimerization.[2] Since DBU is one of the most efficient base for Fmoc-removal,[27] we verified the resin loading only after the coupling with Fmoc-glycine.[28] Furthermore, in order to evaluate the MW assisted synthesis under the 'classical' conditions, we undertook MW reaction also in the presence of pyridine and DMAP both in catalytic and in equimolar amounts (Table 1). As it can be noticed in Table 1, in the case of phenol-like alcohols the use of bases, such as DIPEA and TEA allowed to achieve, in a very short time (30 min) and without the use of toxic reagents, a very high loading (0.380 mmol/g for DIPEA and 0.388 mmol/g for TEA) greater than the loading obtained in the 'classical' way. NMM seemed to be less efficient than the other two tertiary bases: in fact, NMM gave a loading comparable with the literature value (0.246 mmol/g).[11] On the contrary DBU, after the coupling with FmocGlyOH, showed a good loading (0.320 mmol/g), although smaller than that obtained with DIPEA and TEA. Moreover, it is interesting to notice that the use of DMAP and pyridine was detrimental for the immobilization of phenol-like alcohols: in fact, when we employed DMAP, both in catalytic and in equimolar amounts, the loadings were very low (0.120 and 0.064 mmol/g, respectively), while with the use of pyridine no reaction was detected. In the case of Fmoc-ethanolamine, model compound for testing the reactivity of the primary alcohols, the results showed a different behavior than Fmoc-tyramine: in fact the best base seemed to be DBU, which gave, in only 30 min, an excellent loading of 0.560 mmol/g, greater than that obtained with the 'classical' methods.[10,19,11] On the other hand, TEA, DIPEA, and NMM did not appear as good as DBU yielding a loading of 0.204, 0.132, and 0.304 mmol/g, respectively. Contrary to what happened in the case of tyramine, pyridine was effective in MW immobilization of Fmoc-ethanolamine affording a loading of 0.236 mmol/g inferior only to that obtained with DBU and NMM. Conversely, DMAP remained ineffective also with primary alcohols. On the contrary, an interesting result emerged by the immobilization of secondary alcohols, which are extremely problematical to immobilize onto 2CTC: in fact, as it can be noticed from Table 1 MW irradiation only DBU was able to link Fmoc-4-hydroxypiperidine affording a loading of 0.200 mmol/g. Any other conditions tested resulted ineffective.

The effect of the reaction time during MW 2CTC immobilization was investigated planning a series of experiments with different irradiation times (5, 15, 30, 60, and 120 min), Fmoc-tyramine was reacted at constant temperature (50 °C) and concentration (80 mM), using DIPEA and TEA as bases, Fmoc-ethanolamine was

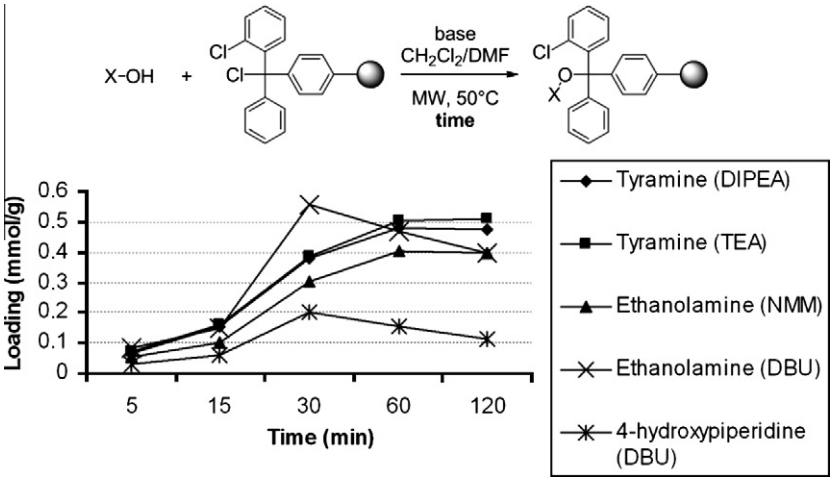


Figure 1. Effect of time in microwave-assisted loading of Fmoc-amino alcohols onto the 2-chlorotrityl resin.

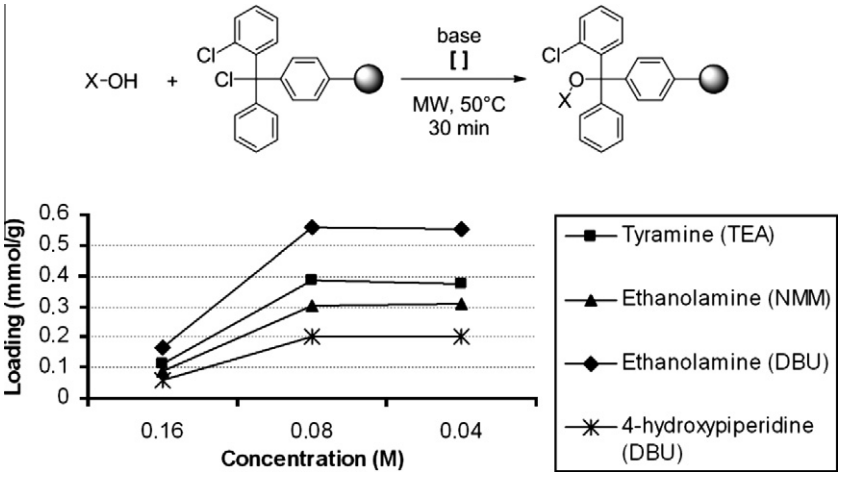
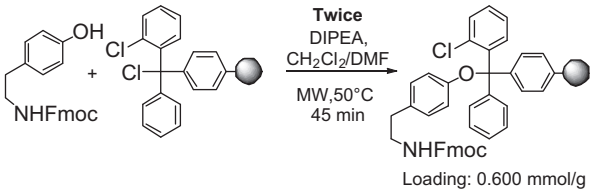


Figure 2. Effect of concentration in microwave-assisted loading of Fmoc-amino alcohols onto the 2-chlorotrityl resin.



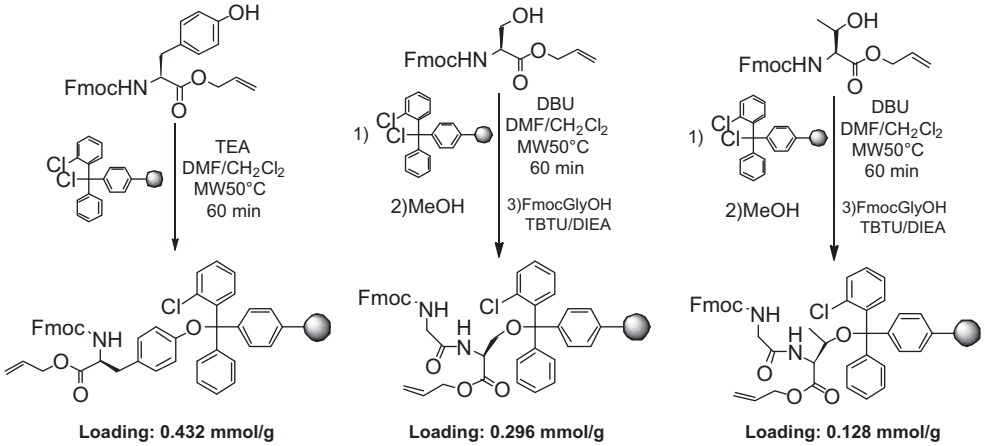
Scheme 2. 'Double coupling' approach.

transformed in the presence of NMM and DBU, while in the case of Fmoc-4-hydroxypiperidine only DBU was used. As shown in Figure 1, when DIPEA, TEA, or NMM were employed, the conversion, both in the case of tyramine and in the case of ethanolamine, reached a maximum value in 60 min, giving loadings of 0.504 mmol/g for Fmoc-tyramine and 0.402 mmol/g for Fmoc-ethanolamine. On the other hand, when DBU was utilized, the maximum loading was obtained at 30 min, both in the case of ethanolamine and 4-hydroxypiperidine, after that time there was a decrease in the loading, a signal of instability of the system.

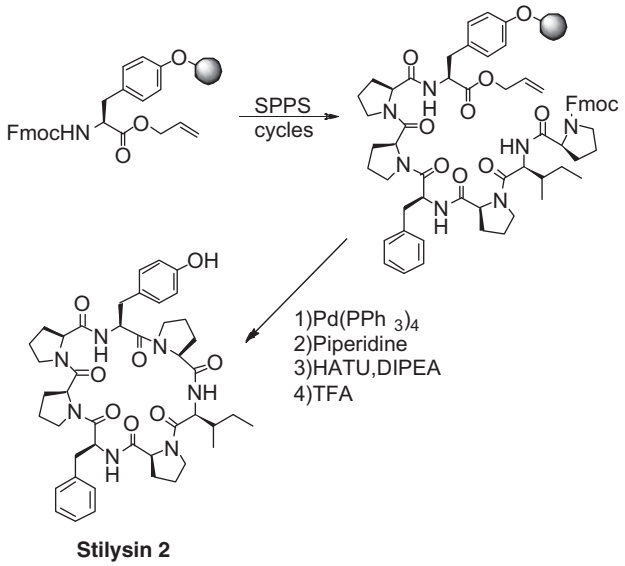
An increase of temperature from 50 to 100 °C (Table 2) did not affect the resin loading, which remained constant both for Fmoc-tyramine, Fmoc-ethanolamine, and Fmoc-4-hydroxypiperidine. Another parameter evaluated was the reaction concentration: as mentioned earlier, the data shown so far refer to reactions with a concentration of 80 mM. So, we tested the MW irradiation of Fmoc-tyramine (with TEA as base), Fmoc-ethanolamine (with NMM and DBU), and Fmoc-4-hydroxypiperidine (with DBU) halving (40 mM) and doubling (160 mM) the concentration, both of the alcohol and of the base. In Figure 2 it can be noticed that the best concentration seemed to be 80 mM: halving the concentration yields a three times lower loading in the cases of tyramine, ethanolamine and 4-hydroxypiperidine, while doubling the concentration did not affect the value. This data can be explained considering the necessity to have a sufficient amount of CH_2Cl_2 in order to maintain the resin swelled.

Finally, in order to further increase the loading of phenol-like alcohols, we carried out a 'double coupling' approach on the resin (Scheme 2): after the first 45 min of reaction of Fmoc-tyramine and DIPEA, the reaction mixture was filtered off and another amount of Fmoc-tyramine and DIPEA was added and stirred again for extra 45 min, reaching a final loading of 0.600 mmol/g, comparable to the loading of secondary alcohols obtained with the use of DBU.

Since 2CTC is widely employed in solid phase peptide synthesis for the immobilization of Fmoc-amino acids,[2,3] we tested the best conditions, found with the model compounds, to bond, through the hydroxyl group, FmocTyrOAll, FmocSerOAll, and FmocThrOAll. This type of immobilization can be useful because it permits to have two growth positions that can be used, for example, for the synthesis of cyclic peptides. The three protected amino acids, synthesized starting from commercially available FmocTyr(*t*Bu)OH,



Scheme 3. MW immobilization of FmocTyrOAll, FmocSerOAll, and FmocThrOAll onto 2CTC.



Scheme 4. Solid phase synthesis of stylisin 2.

FmocSer(*t*Bu)OH, and FmocThr(*t*Bu)OH (see Supplementary data), were subjected to MW irradiation for 60 min at 50 °C in the presence of TEA for tyrosine and DBU for serine and threonine (Scheme 3). Also in the case of the two Fmoc/OAll-protected amino acids the MW irradiation yielded good results, with a loading of 0.432 mmol/g for tyrosine, 0.220 mmol/g for serine, and 0.128 mmol/g for threonine. If we compare these data with those obtained by Bernhardt et al.[11] it can be noticed that we reached the best loading in the case of tyrosine and an equivalent loading in the case of serine and threonine, in a shorter time and without the use of DMAP, a strong nucleophile which can lead to racemization. As proof of concept the synthesis of a cycloheptapeptide characterized by the presence of a tyrosine residue, stylisin 2,[29] was carried out (Scheme 4) immobilizing 60 μmol of FmocTyrOAll onto 2CTC resin and generating the peptide in solid phase following the Fmoc/*t*Bu protocols. After the cyclization step, cleavage and purification of stylisin 2 was obtained in a 13.3% overall yield. In conclusion, we presented a study about the immobilization of primary, secondary, and phenol-like alcohols, onto 2CTC resin by means of MW irradiation: this technique allowed us to achieve the best or comparable loadings than those obtained with the 'classical' methods in a shorter time (30–60 min maximum) and without employing toxic bases, such as pyridine, or racemizing agents, such as DMAP. MW alcohol immobilization was found viable to link to 2CTC, through the hydroxyl group, tyrosine, serine, and threonine, from which it was possible to obtain cyclic peptides.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2011.03.113.

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24. *General procedure for the Fmoc protection*: DIPEA (4 equiv) and then Fmoc-OSu (1.1 equiv) were added to a solution of aminoalcohol (4 mM in CH_2Cl_2). The reaction mixture was stirred overnight at rt. At the end of reaction (TLC monitoring), EtOAc was added and the organic phase was washed with HCl 1 M (2 times), saturated $NaHCO_3$ (2 times), water and brine. The organic phase was dried with anhydrous Na_2SO_4, concentrated in vacuo and the crude product was crystallized from CH_2Cl_2.
25. Deprotection with 20% piperidine in DMF gives the fulvene–piperidine adduct which can be determined by quantitative spectrophotometric analysis at 301 nm (E_{301} = 7800). UV measurements were carried out with a Unicam Heλios α spectrophotometer.
26. *General procedure for microwave-assisted loading of Fmoc-amino alcohols onto the 2-chlorotrityl resin*: Fmoc-amino alcohol (2 equiv) was dissolved in 0.1 ml of DMF and 1.8 ml of CH_2Cl_2 and 2CTC (50 mg of a 1.55 mmol/g; IRIS Biotech), previously swelled with CH_2Cl_2, was added to the reactor. Then the appropriate base (10 equiv) was added and the reaction was stirred in sealed reactor under MW irradiation. At the end of the reaction the excess of reagents was filtered off and the resin was washed with DMF (6 times). To cleave the Fmoc-group, a solution of piperidine (20% in DMF) was added to the resin and the mixture was swirled for 5 min at rt and filtered. Then, another amount of piperidine (20% in DMF) was added and the mixture swirled for other 15 min. The excess of reagents was initially filtered off and successively the resin was washed with DMF (6 times). MW reactions were carried out by Biotage Initiator oven.
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28. At the end of the MW reaction, methanol was added to the mixture, in order to cap the unreacted chloride groups, and the reaction mixture was stirred for 5 min. Then, the excess of reagents was filtered off and the resin was washed with DMF (6 times). A solution of FmocGlyOH (4 equiv), TBTU (4 equiv, 0.45 M in DMF) and DIPEA (10 equiv) was added to the resin and the mixture was stirred for 50 min. At the end of the reaction the excess of reagents was filtered off and the resin was washed with DMF (6 times) and piperidine (20% in DMF) was added to the resin to cleave the Fmoc group.
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