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A protocol for racemization-free loading of Fmoc-amino acids to Wang resin

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Abstract

Fmoc-amino acids are conveniently and inexpensively linked to Wang resin using benzyloxyber zyl chloride resin without the problems normally associated with the traditional methods of preparation of Fmoc-amino acid loa led Wang resins, namely, low substitution, unacceptable racemization or use of expensive reagents.

Since its introduction in 1973,[1] the 4-benzyloxybenzyl alcohol resin (**1**), popularly known as the Wang resin, has been used extensively in solid-phase peptide synthesis with the Fmoc (9-fluorenylmethyloxycarbonyl) protocol.[2] The reasons for its popularity are its stability, low cost and ease of operation and cleavage. The original and traditional method of linking the first Fmoc-amino acid to the resin involves the use of dicyclohexylcarbodiimide and a base, usually 4-dimethylaminopyridine (DMAP). While this method has been satisfactory for simple, non-polar amino acids, unacceptably high levels of racemization are observed when amino acids such as histidine, cysteine, methionine, proline and tryptophan are used.[3] Except in the cases of histidine and cysteine, racemization may be reduced to acceptable levels by using the symmetrical anhydride (5 equiv, or 10 equiv of the Fmoc-amino acid) and reducing the amount of DMAP used.[4] Another well-recognized problem when using DMAP in the above reaction is the formation of dipeptides.[5] To overcome these problems, several strategies have been reported,[6] but another notable disadvantage of low substitution levels remained. Sieber[7] introduced a method that involves the use of a mixed anhydride (5 equiv) of the Fmoc-amino acid and 2,6-dichlorobenzoic acid. More recently, Blakenmeyer et al.,[8] described a method that involves treating the Fmoc-amino acid with 1-(mesitylene-2-sulfonyl)-3-nitro-1*H*-1,2,4-triazole (MSTN) and 1-methylimidazole, but the process is extremely moisture sensitive and expensive. Apart from the methods involving direct esterification of the primary alcohol function noted above, several methods have been introduced to activate the hydroxyl group for linking to Fmoc-amino acids. Notable among these are the use of trichloroacetimidate Wang resin[9] and the Mitsunobu reaction,[10] using diethyl azodicarboxylate and triphenylphosphine. Apart from the expensive reagents, about 10 equiv of the Fmoc-amino acid is used in most of the above methods, which makes the processes very uneconomical.

From the foregoing discussion, it is obvious that the extreme popularity and utility notwithstanding, there is still no satisfactory method of loading Fmoc-amino acids to Wang resin inexpensively and in high loading without racemization. The more obvious methods of converting the Wang resin to the corresponding benzyloxybenzyl chloride[11] and bromide[12] resins and treating them with the cesium salts of Fmoc-amino acids have also been examined but no reliable data is available on the extent of loading that can be achieved with different Fmoc-amino acids under different conditions and the methods are not in common use.

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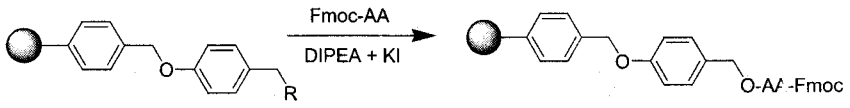

1, R = OH
2, R = Cl

3, AA = Amino Acid

Scheme 1.

We, therefore, examined the various ways of bringing down the cost of loading the Fmoc-amino acids to the Wang resin, which is an important requirement, particularly in commercial syntheses of peptides. In this Letter, we present a protocol for obtaining Fmoc-amino acid-linked Wang resins rapidly, inexpensively and with near quantitative loading, avoiding the use of expensive reagents including cesium salts (Scheme 1). Racemization, even with histidine derivatives is undetectably low. The method involves converting the benzyloxybenzyl alcohol group of the Wang resin, **1**, into a benzyloxybenzyl chloride group using thionyl chloride[13] and treating the Wang chloride resin thus obtained with the Fmoc-amino acid and a non-nucleophilic base such as diisopropylethylamine (Hunig's base) in dimethylformamide (DMF) at room temperature; about 10 mol % of potassium iodide is used to accelerate the reaction. The protocol described in this paper, which is characterized by its simplicity in comparison to the above cited methods, was arrived at after experimenting with several variations of the Fmoc-amino acid, the solvent, the metal salt, the base and their relative proportions.

Typically, the protocol involves swelling the Wang chloride resin (**2**, 1 g; ~1 mmol) in DMF (10 ml), reacting it with the Fmoc-amino acid (3 mmol), potassium iodide (0.3 mmol) and diisopropyethylamine (3 mmol) under stirring for 16–24 h at room temperature.[14] The loading of the Fmoc-amino acid on the resin, **3**, was determined by both weight gain and by spectrophotometry[2] and the results with different representative Fmoc-amino acids are summarized in Table 1. As can be seen, the loading, even for more complex Fmoc-amino acids, was much higher than most of the methods reported in the literature. The first DMF filtrate on dilution with water (1:10) and acidification resulted in the recovery (~75% after purification) of the unreacted (excess) Fmoc-amino acid that could be reused. The process was conveniently scaled up to 1 kg level.

Table 1
Results of loading of different Fmoc-amino acids and peptides by the described method

Fmoc-amino Acid	Reaction time (h)	Fmoc loading by weight gain (mmol/g)	Fmoc loading by assay (mmol/g)
Fmoc-Asp(O-*t*Bu)OH	20	0.49	0.51
Fmoc-His(Trt)OH	20	0.49	0.50
Fmoc-Asn (Trt)OH	24	0.50	0.52
Fmoc-MetOH	16	0.55	0.60
Fmoc-ValOH	16	0.57	0.59
Fmoc-Trp(Boc)OH	24	0.52	0.53
Fmoc-Val-PheOH	20	0.47	0.48

In view of the very mild (room temperature) and near-neutral conditions employed, racemization during the linking process was considered unlikely. Nevertheless, this was confirmed to be the case by HPLC analysis of the dipeptide, Fmoc-Val-His(Trt)OH prepared by standard protocols.[15]

The applicability of the above protocol for, (a) synthesis of peptides, and (b) for linking protected peptides to Wang resin was demonstrated by preparing Fmoc-Val-PheOH by standard protocols using Fmoc Phe-O-Wang resin made by the present method and linking it again to Wang resin using the present protocol.[16]

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14. The reaction was monitored by withdrawing a few mg of the resin, washing and drying the resin (see below) and estimating the remaining chlorine on the resin by the Volhardt method as well as spectrophotometrically (by Fmoc-release assay). At the end of the reaction, the resin was filtered and washed successively with DMF (5 ml), methanol (5 ml), DMF (2 × 5 ml) water (2 × 10 ml), methanol

(5 ml), dichloromethane (2 × 10 ml), and methanol (2 × 5 ml) and then dried.

15. Fmoc-L-His(Trt)-Wang resin prepared according to the above protocol was treated with 20% piperidine in DMF to deblock the amino group and then treated with the ester of Fmoc-L-valine and 1-hydroxybenzotriazole to obtain Fmoc-Val-His(Trt)O-Wang resin. The dipeptide, Fmoc-L-Val-L-His(Trt)OH was released from the resin using DCM–TFA (1:1) and analyzed by HPLC; no peak for Fmoc-L-Val-D-His(Trt)OH was detectable.

16. Fmoc-Phe-O-Wang resin (0.56 mmol/g), prepared as per the present protocol was treated with 20% piperidine in DMF for 1 h and the resultant deprotected Phe-O-Wang resin was added to a solution of Fmoc-Val-O-1-hydroxy-benzotriazole ester in THF prepared by standard protocols (Ref. 2). The peptide thus formed was cleaved from the resin using DCM–TFA (1:1), yielding Fmoc-Val-PheOH, quantitatively, and in 100% purity (HPLC). The dipeptide was then linked to Wang chloride resin using the present method, with satisfactory results (0.48 mmol/g Fmoc-peptide substituion; Table 1).