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A CONVENIENT SOLID PHASE PREPARATION OF PEPTIDE SUBSTITUTED AMIDES

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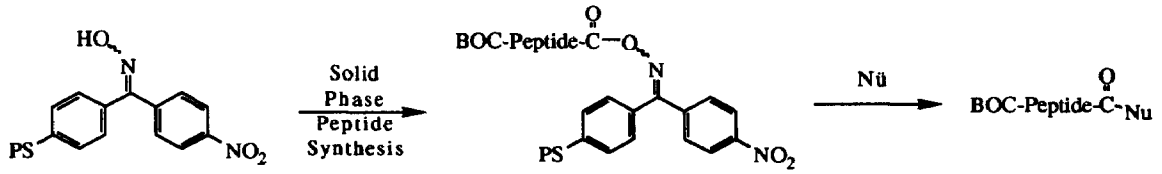
Abstract: The preparation of the title compounds by a solid phase procedure is reported. The method involves the incorporation of different amines at the C-terminal position of a peptide molecule by its nucleophilic displacement from the oxime resin. The method works well with primary aliphatic and aromatic amines but gives lower yields with deactivated or sterically hindered amines.

The preparation of peptide substituted amides is highly desireable for several purposes. The introduction of various alkyl- and aryl amines at the C-terminal position of a peptide can enhance its biological activity and proteolytic stability. On the other hand, peptide substrates having a chromophoric or fluorescent group at the carboxyl end are useful biochemical probes to monitor the activity of numerous enzymes. Most of the methods to prepare C-terminal amides involve conventional solution reactions between the peptides and the desired amines and they are synthetically tedious. A solid phase strategy which allows the amine to be introduced at the carboxyl end would be highly advantageous.



1 PS = Polystyrene

Scheme 1

However, to date, reports on that subjects are very scarce[1] since most of the resins commonly used in solid phase peptide synthesis are designed to give peptide acids or primary amides after a hydrolytic cleavage.[2] By contrast, the oxime resin **1** developed by Kaiser and DeGrado[3] is probably the most suitable resin to develop a general solid

phase synthesis of peptide substituted amides. Indeed, that resin allows the preparation of peptides using the BOC strategy and their subsequent cleavage from the support by a nucleophilic displacement at the carboxyl terminus. This leads to the incorporation of the nucleophile at that position (Scheme 1). Nucleophiles reported so far in the cleavage reaction include aminoacid and peptide esters,[3,4] hydrazine,[3,4] ethylamine,[5] cyclohexylamine,[5] 2-(aminomethyl)pyridine,[5] hydroxy-piperidine,[3,4] tetrabutylammonium salt of amino acids,[6] and 2,4-dinitroanilino-ethylamine.[7]

Table 1. Nucleophilic cleavages of BOC-Ala$_3$-COO-N=Oxime resin[8] by different amines.[a]

Nucleophile	Cleavage conditions	Work up procedure[b]	% Cleavage[c]	Isolated yield (%)
n-Propylamine	1h, $CHCl_3$	1	91	89
	4h, $CHCl_3$	1	99	91
Dodecylamine[d]	4h, $CHCl_3$	1	77	76
Benzylamine	4h, $CHCl_3$	2	97	87
Diethylamine	48h, $CHCl_3$ 2% CH_3COOH	2	60	35
Aniline	24h, $CHCl_3$	2	24	10
	24h, $CHCl_3$, 2% CH_3COOH	2	95	81
4-Nitroaniline[e]	48h, $CHCl_3$/DMF(4/1)	2	<5	---[f]
	48h, $CHCl_3$/DMF(4/1) 2% CH_3COOH	2	30	16
7-Amino 4-methyl-coumarin[e]	48h, $CHCl_3$/DMF(4/1)	2	<5	---[f]
	48h, $CHCl_3$/DMF(4/1) 2% CH_3COOH	2	<5	---[f]

a) Typical procedure: 300 mg of BOC-Ala$_3$-Resin (0,34 mmol/g) was treated with the nucleophile solution and mechanically shaken. (b) Work up procedures: 1)the cleavage solution was filtered and the resin washed with a $CHCl_3$/MeOH (3/1) solution. The combined solutions were evaporated to dryness, dried for 2 h under high vacuum, then triturated with ether; 2) same as procedure 1, but the crude mix was dissolved in CH_2Cl_2, washed with twice with 0.5N HCl, 5% $NaHCO_3$, and H_2O, then dried with anhydrous $MgSO_4$, filtered, and evaporated. (c) Determined by the quantitative ninhydrin test[9] on the resin before and after the cleavages. (d) To simplify the work up, only one equivalent of dodecylamine was used instead of a 0.5 M solution. (e) A saturated solution of the nucleophile in $CHCl_3$/DMF (4/1) was used (≈0.1 M). (f) No product was isolated.

Here we report the results of a study on the scope and limitations of this solid phase method to prepare peptide substituted amides. We have investigated the nucleophilic cleavage of a tripeptide, N-BOC-Ala-Ala-Ala, from the oxime resin using several nucleophiles that could provide useful compounds. The results are reported in Table 1. All the cleavages were performed in $CHCl_3$ at room temperature using a 0.5 M solution of the nucleophiles except in the cases of 4-nitroaniline and 7-amino 4-methyl coumarin where saturated $CHCl_3$/DMF (4/1) solutions (around 0.1 M) were used. When primary alkyl amines were used, the cleavages were completed after 4 h and the peptide amides were isolated in high yields. The use of a sterically hindered secondary amine, $HNEt_2$, lead to 60% of cleavage, but the desired peptide amide was isolated in 30% yield. Cleavage with the less nucleophilic aniline gave the desired peptide anilide

Table 2. Protected peptide n-propylamides prepared by nucleophilic cleavage with n-propylamines.[a]

Peptides	Yield[b]
BOC-Phe-Ala-NH-n-C_3H_7	95%
BOC-Ala-Ala-Ala-CE-Ala-CE-Ala-NH-n-C_3H_7 [c]	86%
BOC-Leu-(OBzl)Glu-Ala-Leu-Phe-(OBzl)Glu-Ala-NH-n-C_3H_7	48%
BOC-Leu-(OBzl)Glu-Ala-Leu-Phe-Ala-Leu-Phe-(OBzl)Glu-Ala-NH-n-C_3H_7	32%

(a) All peptides were cleaved by a 4h treatment with a 0.5 M solution of n-propylamine in $CHCl_3$. After crystallization from a $CHCl_3$/MeOH solution, they were characterized by ^1H NMR and FAB mass spectrometry. (b) Pure isolated yields. (c) CE= 3,4-(18-crown-6) L-Phenylalanine.[10]

with an 81% isolated yield but the reaction was slower, even in the presence of acetic acid , requiring 48h for completion. The use of the deactivated 4-nitroaniline lead nonetheless to 30% of cleavage and to the obtention of 16% of the peptide nitroanilide.[11] However, no reaction was observed with 7-amino 4-methyl coumarin. The lower cleavage yield in these last two cases is probably related to the weak nucleophilic character of these amines, but can also be due in part to the lower concentration of the nucleophiles. On the other hand, no racemization could be detected by HPLC and ^1H NMR in all the cleavage reactions.

To verify the applicability of the method to longer peptides, we have synthesized the peptides listed in Table 2 on the oxime resin and cleaved them with n-propylamine. As seen from the table, the cleavage proceeds well with longer peptides

having different functionalities. In particular, with peptides having one or two benzyl protected glutamic acids, it is noteworthy that the cleavage reactions proceed cleanly without any aminolysis of the side chains confirming a previous report[5].

In summary, the nucleophilic cleavage of peptides attached to the oxime resin is a versatile method to prepare protected peptides substituted amides. The procedure is rapid, simple, and works well with primary aliphatic amines and aromatic amines that are not deactivated. However, poor nucleophilic or sterically hindered amines do not react under the conditions used. The preparation of other useful peptide C-terminal derivatives by this solid phase procedure is currently underway in our laboratory.

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