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Direct synthesis of *N*-protected β-amino dimethylhydroxamates: Application to the solid-phase synthesis of a peptide incorporating a new amide bond surrogate Ψ[CH_2CH_2NH]

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Abstract.

A rapid and efficient one-step synthesis of N-protected β-amino dimethylhydroxamates starting from diazo ketones is reported. A Fmoc-protected β-amino aldehyde obtained by reduction of its corresponding dimethylhydroxamate was incorporated during solid phase assembly of an antigenic peptide. The resulting pseudopeptide containing an ethylene amino bond Ψ[CH_2CH_2NH] was efficiently recovered.

Keywords: Peptide analogues/mimetics; Solid-phase synthesis; Rearrangements.

During the last years, the interest for β-amino acids has increased considerably. β-amino acids, though less abundant than α-amino acids, are components of some natural peptides and recent structural studies have shown that β-peptides can fold into well-defined and stable helical or pleated-sheet secondary structures [1-5]. Moreover, β-peptides are more resistant to enzymatic degradation [6]. On the other hand, the reduced amide bond has been intensively used in the design of peptidase inhibitors and bioactive peptides [7, 8]. It was tempting to combine these two modifications for the solid-phase synthesis of pseudopeptides with reduced peptide bond and involving β-amino acids, giving access to a new type of amide bond surrogate that we propose to call ethylene amino bond Ψ[CH_2CH_2NH].

The key intermediates are the *N,O*-dimethylhydroxamates of β-amino acids, which are also extremely useful in pseudopeptide chemistry for the synthesis of carba bond surrogate Ψ[CH_2-CH_2] [9]. Generally, *N*-protected β-amino acids are first prepared *via* the Wolff rearrangement of diazo ketones from commercially available α-amino acids [10]. They are then converted to the corresponding dimethylhydroxamate by reaction with *N,O*-dimethylhydroxylamine hydrochloride, in the presence of an activating agent.

To avoid this time-consuming procedure, we report here the straightforward rearrangement of α-amino diazo ketones with *N,O*-dimethylhydroxylamine as a nucleophile. Several studies have shown that the Wolff rearrangement of *N*-protected α-amino diazo ketones could be done in the presence of water, methanol, methylamine, nucleosides or amino esters to obtain

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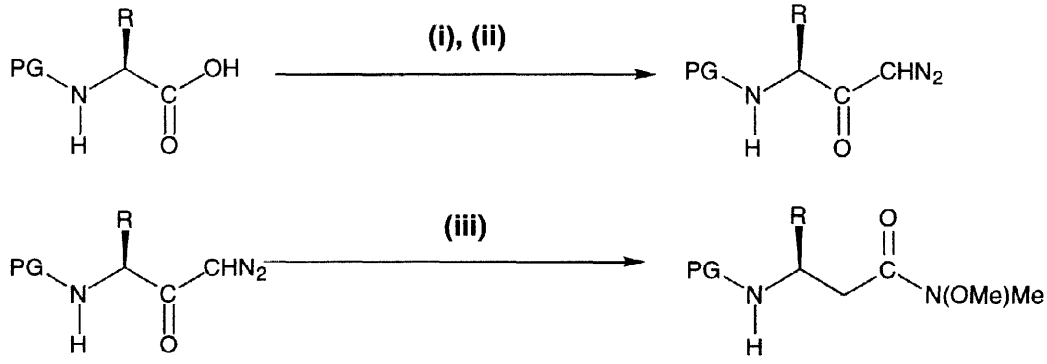
respectively the β-amino acids, -esters, -amides or -peptides with retention of configuration [11-13]. A preliminary test involving the simultaneous addition of the *N,O*-dimethylhydroxylamine hydrochloride and triethylamine to a mixture of *N*-protected diazo ketone and silver benzoate gave very poor yields (20-30%). In a second attempt, hydroxylamine hydrochloride was regenerated separately with triethylamine in THF overnight at room temperature due to the poor solubility of the hydrochloride. After filtration, the solution was directly used for the rearrangement. The steps leading to the *N*-protected β-amino dimethylhydroxamates are described in scheme 1.



Scheme 1.
(i) 1.1 eq. iBuOCOCl/NMM, THF, -25°C, 1 h; (ii) CH_2N_2/Et_2O, r.t., 2 h; (iii) 1.5 eq HN(OMe)Me/2 eq. Et_3N/THF filtrate, 3 eq. Et_3N, 0.15 eq. $C_6H_5CO_2Ag$, THF, -25°C to r.t. in 2 h. PG: protecting group, R: side chain.

This procedure was applied successfully to several Boc protected α-amino acids with different side chain fonctionalizations and to some Fmoc amino-acid derivatives (Table 1). *N*-Boc-protected diazo ketones were clearly converted to the corresponding hydroxamates in excellent yields (84-96%).

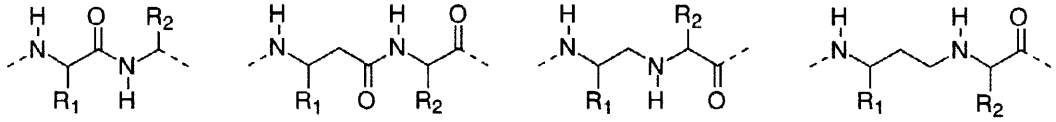
In the case of Fmoc derivatives, the final yield was at least 92%, indicating the possibility to obtain Fmoc-protected β-amino dimethylhydroxamates even if the *N*-terminal protecting group was exposed to a basic medium (Et_3N) during several hours.

N-protected amino acid	*N*-protected diazo ketone (%)[1]	*N*-protected β-amino dimethylhydroxamate (%)[2]
Boc-Ala-OH	93	96
Boc-Val-OH	86	90
Boc-Phe-OH	95	94
Boc-Ile-OH	91	85
Boc-Leu-OH	98	87
Boc-Thr(Bzl)-OH	92	84
Boc-Tyr(Bzl)-OH	95	88
Boc-Lys(2-Cl-Z)-OH	98	94
Boc-Ser(OBzl)-OH	97	97
Fmoc-Phe-OH	96	94
Fmoc-Ser(OtBu)-OH	94	92

[1] Conversion of the protected α-amino acids into the *N*-protected diazo ketones.
[2] Direct rearrangement of the diazo ketones into the corresponding dimethylhydroxamates.
Yields are calculated after purification by flash chromatography in ethyl acetate/hexane (typically 1:1).

Table 1
Conversion of *N*-protected α-amino acids into the corresponding *N*-protected dimethylhydroxamates.

We then decided to introduce the $\Psi[CH_2CH_2NH]$ modification (d in Fig.1) in a model peptide, which spans the major antigenic site of foot-and-mouth disease virus (FMDV), serotype O1 corresponding to the region 147-156 of the viral protein 1 (VP1). This peptide (Ac-CDFGSLAPRVA-OH) has been recently shown to induce virus-neutralizing antibodies in guinea pigs [14]. Based on our observation that enzyme cleavage occurs mainly between Ser^{150} and Leu^{151}, we decided to study isosteric replacements in this region. A pseudopeptide containing a reduced peptide bond $\Psi[CH_2NH]$ (c in Fig.1) at the same position in the sequence and a peptide analogue containing a β-HSer residue (b in Fig.1) were synthesized in parallel.



a b c d

Figure 1.
a: natural peptide bond; b: β-amino acid involved in a peptide bond; c: reduced peptide bond; d: β-amino acid involved in a reduced peptide bond.

Peptides were assembled on a Wang resin using a multichannel synthesizer employing a standard Fmoc protocol, and the peptide chains were elongated up to the Leu^{151} position [15]. The Fmoc-protected α- and β-amino dimethylhydroxamates were then reduced with $LiAlH_4$ to afford us the desired aldehydes with satisfactory yields [16]. The methylene and ethylene amino bonds were finally introduced by reductive alkylation between the amino group of the resin-bound peptide and the Fmoc-protected α- and β-amino aldehydes respectively [17]. The *N*-Fmoc protected β-HSer derivative was prepared in 75% yield by using the modified Wolff rearrangement as described recently by Leggio *et al.* and was then incorporated on the growing peptide chain [18]. After Fmoc removal, the elongation of the peptides was achieved classically.

Compound	Peptide Content in Crude Product (%)[a]	Purity (%)[b]	Overall yield (%)[c]
Ac-CDFGβSLAPRVA-OH	84	97	51
Ac-CDFGSΨ[CH_2NH]LAPRVA-OH	73	97	25
Ac-CDFGSΨ[CH_2CH_2NH]LAPRVA-OH	71	98	22

a. Determined by analytical RP-HPLC.
b. Products were purified by semi-preparative RP-HPLC and gave satisfactory RP-HPLC, MS results.
c. Calculated using the initial substitution level of the Wang resin.

Table 2
Peptide and pseudopeptides prepared by SPPS

The three resin-bound peptides were cleaved using the King's reagent [19]. After cleavage, each pseudopeptide was purified by HPLC (the final purity of each product was at least 97%) and MALDI-TOF analysis gave the expected values[1].The results shown in Table 2 suggest that

1. RP-HPLC was performed on an analytical HPLC Beckman instrument (Gagny, France) with a nucleosil 5 mm C18 column (3.9 x 150 mm) using a linear gradient of (A): 0.1 % (v/v) TFA in water and (B): 0.08% (v/v) TFA in acetonitrile, at a flow rate of 1.2 mL/min with UV detection at 214 nm. Peptide analogues were analyzed on a linear PROTEIN TOF instrument (Bruker Spectrospin, Bremen, Germany)

the pseudopeptide containing a β-amino acid involved in a reduced peptide bond $\Psi[CH_2CH_2NH]$ can be prepared as efficiently as the $\Psi[CH_2NH]$ analogue without particular increase of dialkylation during the reductive amination.

In conclusion, we have demonstrated that the preparation of *N*-protected dimethylhydroxamates *via* the direct Wolff rearrangement is an easy process. All appropriately protected α-amino acids could be used in this method. After reduction of the corresponding *N,O*-dimethylhydroxamates, *N*-protected β-amino aldehydes can be efficiently incorporated during solid-phase assembly and enable the generation of a new family of pseudopeptides.

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