Regioselective oxyfunctionalization of peptides by dimethyldioxirane: tertiary C–H σ-bond oxygen atom insertion into leucine derivatives and leucine-containing dipeptides

Maurizio Mezzetti, Enrico Mincione* and Raffaele Saladino*†

Dipartimento Agrochimico Agrobiologico, Università degli studi di Viterbo 'La Tuscia', via San Camillo de Lellis, 01100 Viterbo, Italy

A simple and straightforward approach to selective C–H σ-bond oxygen atom insertion into Leu residues on peptides using dimethyldioxirane is described; this procedure is compatible with Gly, Ala, Val, Ile and Phe residues.

Proteins with altered functions can be prepared from existing molecules by genetic manipulations[1] or, particularly in the case of small peptides, by chemical methods, *via* side-chain alteration and backbone modifications. Several chemical methods for performing side-chain alterations have been reported. The tryptophan side-chain is efficiently transformed by Ru^{VIII}, generated *in situ* from $RuCl_3$ and $NaIO_4$, to that of aspartic acid.[2] A Ru^{VIII} reagent has subsequently been used to oxidize 13 to the 20 coded α-amino acids[3] and for a novel protein backbone modification by C(α)–C side-chain scission.[4] Moreover, selective modification of serine- and threonine-containing peptides has been accomplished with lead tetraacetate to give α-acyloxyglycine derivatives, which can be easily transformed into α-alkylthio- and α-halogeno-glycines.[5] Recently, a simple approach to selective iodination of tyrosine residues on peptides using IPy_2BF_4 (Py = pyridine) has been described,[6] and preliminary studies revealed that the method might be also suitable for iodination of phenylalanine derivatives.

In spite of extensive work on side-chain transformations of aromatic, heterocyclic and heteroatom moieties in peptides, no general methods have been described for oxygen atom insertion into the C–H σ-bond present in the side-chain of α-amino acids, with the exception of the glycine-selective chemical modification by Nickel peroxide,[7] in which case a concomitant backbone modification was observed, probably because of the low reactivity generally shown by this bond.

A powerful and selective oxidant for this purpose,[8] which performs under strictly neutral conditions, is dimethyldioxirane (DMD). The advantages of dioxiranes, especially DMD, in synthetic chemistry have been well documented in alkene epoxidations, heteroatom oxidations and C–H σ-bond insertions. To the best of our knowledge, DMD has been used in the chemistry of α-amino acids and peptides to perform the oxidation of α-diazo ketones derived from α-amino acids,[9] and in the synthesis of enantiomerically pure N-protected β-amino-α-keto esters from α-amino acids and dipeptides.[10] In each case, the possible oxygen atom insertion into the side-chain was not observed. We report here a simple, selective and straightforward approach to the C–H σ-bond oxygen atom insertion into Leucine (Leu) and Leu-containing dipeptides.

The oxidation of Boc-Leu-OMe **1** (1 mmol) performed with a freshly prepared solution of DMD (0.09 mol dm^{-3} acetone solution, 6.0 equiv.)[11] in CH_2Cl_2 (5 cm^3) at 25 °C for 3 days afforded the 4,4-dimethyl-4-butanolide derivative **2** as the only recovered product in 42% isolated yield (Scheme 1),‡ with some unreacted substrate. Compound **2** is probably formed by selective oxygen atom insertion into the tertiary C–H σ-bond of Leu followed by cyclization.

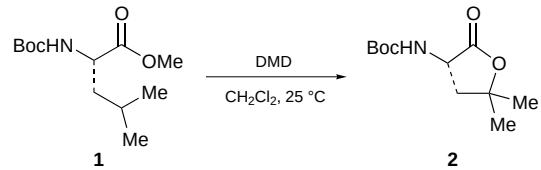
Some other protected α-amino acids bearing an alkyl side-chain (Boc-Gly-OMe, Boc-Ala-OMe, Boc-Val-OMe, Boc-Ile-OMe and Boc-Phe-OMe) were then studied. The reactions performed with DMD (0.09 mol dm^{-3} acetone solution) in CH_2Cl_2 (5 cm^3) at 25 °C for 3 days did not give products in appreciable amounts, even in the presence of a large excess of DMD and after longer reaction times.

It is known that the DMD oxygen atom insertion into the C–H σ-bond shows a pronounced tertiary–secondary–primary selectivity.[8] Furthermore, this transformation is extremely sensitive to solvent[12] and stereoelectronic effects. In particular, a dipolar group close to the reactive centre may influence the reaction, favouring a chemo- and stereo-specific attack,[13] or preventing the oxidation.[14]

We can put forward only hypotheses to explain the unexpected stability of the valine substrate in the DMD oxidation. A rationalization based on simple electronic deactivation by the amino functionality on the more proximate tertiary C–H σ-bond with respect to leucine seems reasonable. This hypothesis is in accord with the recently reported procedure[15] for the selective oxyfunctionalization of unactivated C–H σ-bonds of alkylamines in acid medium by methyl(trifluoromethyl)dioxirane, in which the strong electron-withdrawing nature of the ammonium group deactivates the oxidation of even tertiary C–H σ-bonds at the α and β positions. In our case, the reaction appears to be operative only when a tertiary C–H σ-bond is in the remote C(γ) position with respect to the amino acid functionality.

The feasibility of transformation of **1** by DMD with respect to the other α-amino acids bearing an alkyl side-chain promped us to evaluate the possibility to obtain a chemo- and regio-selective modification of Leu-containing dipeptides. The oxidation of Boc-Ala-Leu-OMe **3** and Boc-Leu-Ala-OEt **5** with an excess of DMD in CH_2Cl_2 (5 cm^3) at 25 °C for 3 days afforded the butanolide derivate **4**§ and the peptide derivative 6¶ in 35 and 38% isolated yield, respectively, plus some unreacted substrates (Scheme 2). Compound **4**, which is obtained by selective modification of the Leu residue, may be formed by selective oxygen atom insertion into the tertiary bond present in the side-chain of Leu, followed by cyclization. On the other hand, the presence of a free alcoholic moiety in **6** (as shown by the OH group absorption at 3390 cm^{-1} in the IR spectrum) suggests that, in this case, a spontaneous cyclization process is not operative. This hypothesis was further confirmed by the absence of a detectable amount of the butanolide derivative **2** in the reaction mixture.

Finally, we studied the oxidation of the dipeptide Boc-Leu-Leu-OMe **7** by DMD under similar experimental conditions.[16]



1 **2**

Scheme 1

The oxidation of **7** with DMD (0.09 mol dm^{-3} acetone solution, 6.0 equiv.) in CH_2Cl_2 (5 cm^3) at 25 °C for 3 days afforded the butanolide derivative **8** in 33% isolated yield (Scheme 3).‖ Minor amounts of **9** were also formed, as shown by ^1H and ^{13}C NMR spectral analyses of the crude reaction mixture prior to purification.

These data show that the reactivity of the Leu residue towards DMD is extremely sensitive to the position (C/N-terminal position) of the residue into the peptide. In particular, C-terminal Leu appears to be more reactive than the N-terminal residue towards DMD. In this case, steric factors may operate, since dioxiranes are generally sensitive to steric hinderance,[8] even if electronic deactivation by the two amino acid functionalities of the Leu N-terminal residue cannot be ruled out.

The high selectivity observed for the Cγ–H σ-bond of Leu towards DMD offers a novel synthetic route to modified peptides without backbone modifications. Work is in progress in our laboratories to study the reactivity pattern of the other coded α-amino acids towards DMD oxidation, as well as to transform the newly introduced hydroxy moieties into other useful functionalities.

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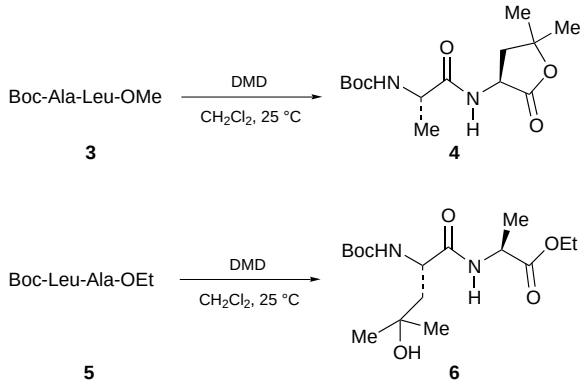
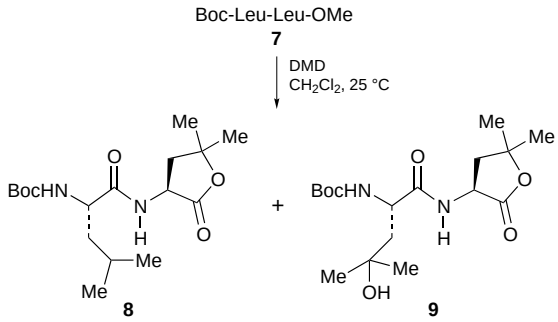


Scheme 2



Scheme 3

Footnotes

† E-mail: saladino@unitus.it

‡ Compound **2** was purified by flash-chromatography. MS analysis for **2** (ESI-MS) shows *m/z* 229 $[M]^+$, in agreement with the proposed structure.

§ Compound **4** was purified by flash-chromatography. MS analysis for **4** (ESI-MS) shows *m/z* 299 $[M + H]^+$, in agreement with the proposed structure.

¶ Compound **6** was purified by flash-chromatography. MS analysis for **6** (ESI-MS) shows *m/z* 334 $[M]^+$, in agreement with the proposed structure.

‖ Compound **8** was purified by flash-chromatography. MS analysis for **8** (ESI-MS) shows *m/z* 330 $[M]^+$, in agreement with the proposed structure.

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