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A novel oxidative side-chain transformation of α-amino acids and peptides by methyltrioxorhenium/H_2O_2 system

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Abstract—*N*-Boc derivatives of Met, Cys, and Trp, the properties of which resemble those of the respective amino acid residues present in proteins, are efficiently oxidized by methyltrioxorhenium and H_2O_2. A high regioselectivity for the oxidation of these residues when embedded into peptides was also found.
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The synthesis of unnatural amino acids and peptides offers great flexibility for the designing of novel bio-active protein analogues.[1] Several methods have been developed in recent years to overcome some drawbacks, such as the poor stability and the lack of oral absorption, which can reduce the use of peptide analogues as therapeutic agents. Two main synthetic strategies based on amino acid side-chain transformations and backbone modifications were proposed. Backbone modifications include changes at any one of the three characteristic repeating NH, CO, and α-CH elements.[2] Side-chain modifications are mainly obtained by oxidation of low redox potential residues.[3–8] Selective C–H hydroxylations of high redox potential leucine derivatives have been also described.[9a] Despite extensive work on stoichiometric procedures for amino acid side-chain modifications,[9b] only a few examples concerning the use of catalytic procedures are reported. In the last decade methyltrioxorhenium (MTO, $MeReO_3$) has been used in several organic transformations.[10] The reactive intermediates for these oxidations are a monoperoxo [$MeRe(O)_2O_2$] and a bis-peroxo [$MeReO(O_2)_2$] η^2-rhenium complexes.[11] To the best of our knowledge there are no reports in the literature dealing with the oxidation of amino acids and peptides with MTO. Herein we describe that *N*-Boc derivatives of methionine (Met), cysteine (Cys), and triptophane (Trp), the properties of which resemble those of respective amino acid residues in proteins, are efficiently oxidized by MTO and environmental friendly H_2O_2. Noteworthy, a high chemoselectivity was observed in the oxidation of these amino acids when embedded into peptides.

Initially, we tried to study the oxidation of *N*-Boc derivatives of Val, Leu, Ile, Pro, Ser, Tyr, Thr, Met, Cys, His, and Trp as representative model compounds. Briefly, 1.0 mmol of substrate dissolved in 5 mL of solvent (EtOH or AcOH), was added portionwise with MTO (5% in weight) and H_2O_2 (30% aqueous solution; 2.0 equiv of H_2O_2 except where otherwise specified) at room temperature. At the end of reaction a small amount of MnO_2 was added and the solvent evaporated after filtration. The reaction products were characterized by the usual NMR and MS analyses and by comparison with authentic samples.[1c] Under these experimental conditions MTO showed a high chemoselectivity, Boc-Met-OMe **1**, Boc-Cys-OMe **2**, and Boc-Trp-OMe **3** being the only reactive substrates. Treatment of **1** with MTO/H_2O_2 system in EtOH afforded after 2 h the corresponding sulfone **4** as the only recovered product, in quantitative conversion of substrate and 80% isolated yield (Scheme 1). A better result was obtained in acetic acid, in which case **4** was recovered in 90% yield after 10 min.

The oxidation of **2** in EtOH was performed with different amounts of H_2O_2. In the presence of 2.0 equiv of

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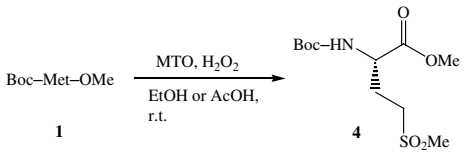
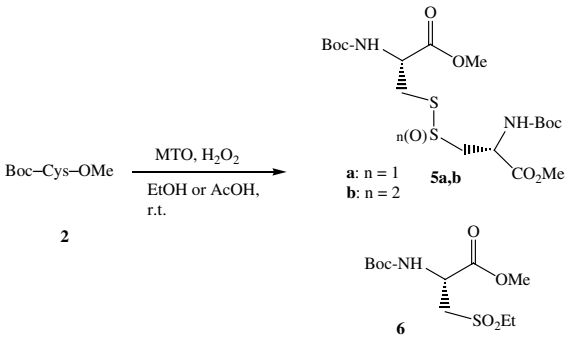


Scheme 1.

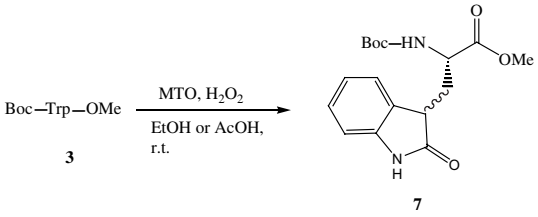
oxidant, the sulfoxide **5a** was recovered as the main product (48%) beside to sulfone **5b** (23%) and the sulfinic ester **6** (22%), probably formed by oxidative sulfur–sulfur bond cleavage (Scheme 2). Sulfoxide **5a** became the main product (75%) when the reaction was repeated with 1.0 equiv of H_2O_2, cystine (not shown), and **5b** being recovered only in a small amount (8% and 10%, respectively). A different reaction pattern was obtained in acetic acid (2.0 equiv of H_2O_2), in which case **5b** was recovered in 70% yield along with cystine (10%).

Selective oxidation of sulfur containing amino acids is an important tool for the chemical engineering of proteins. For example, the oxidation of methionine residues has been used as a probe for the determination of binding domains of proteins on lipidic surfaces.[4a] Moreover, the oxidation of cysteine moieties to corresponding sulfoxides or sulfones resulted in a significant improvement of the activity in several human immunodeficiency virus (HIV) protease inhibitors.[3]

The oxidation of **3** in EtOH afforded the methyl *N*-(*tert*-butoxycarbonyl)-3-(2-oxo-2,3-dihydro-1*H*-indol-3-yl)alaninate **7** (Scheme 3) (chromatographically separable 1:1 mixture of diastereomers) in quantitative conversion of



Scheme 2.



Scheme 3.

substrate and 90% yield. Again, the reactivity of MTO increased in acetic acid, in which case **7** was rapidly obtained in quantitative yield.

It must be stressed that the reactivity and selectivity of MTO in the oxidation of Met, Cys, and Trp appear to be similar to that previously found for the myeloperoxidase/H_2O_2 system.[12]

The feasibility of transformation of Met, Cys, and Trp derivatives with respect to other amino acids bearing aliphatic and aromatic side-chains, prompted us to investigate the chemo- and regio-selective modification of peptides. Oxidations were performed in EtOH under the conditions used for simple amino acids. The products were characterized by NMR and liquid chromatography–mass spectrometry/mass spectrometry (LC–MS/MS) analyses. Table 1 shows selected ESI/MS data for functionalized peptides.

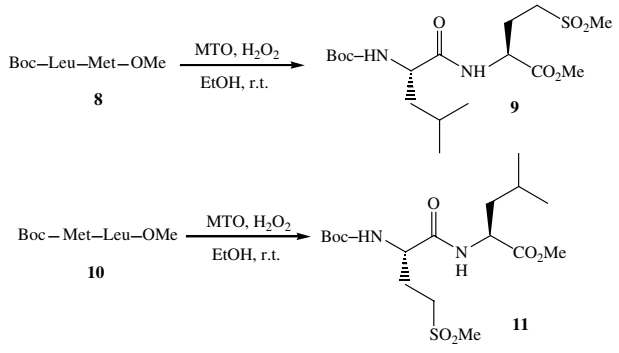
In accordance with results previously obtained in the transformation of **1**, the oxidation of Boc-Leu-Met-OMe **8** afforded the sulfone **9** as the only recovered product in 90% yield (Scheme 4).

In a similar way, Boc-Met-Leu-OMe **10** afforded the sulfone **11** in 83% yield (Scheme 4). Thus, the position (*N*-terminal versus *C*-terminal) of Met in the peptide was irrelevant on the pattern of the reaction.

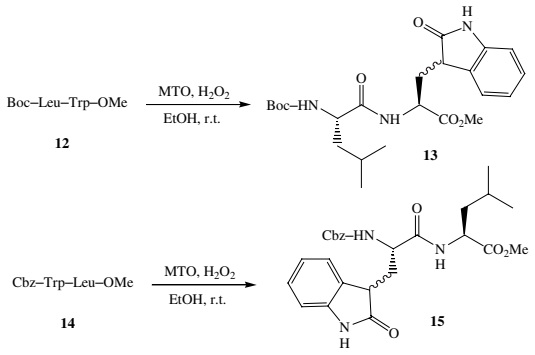
Table 1. Selected ESI/MS data for functionalized peptides

Product	MS: (M+1)[a]	MS/MS[b]
9	409	—
11	409	—
13	448	—
15	482	—
17	466	366, 332, 248
18	482	382, 318, 235
20	500	468, 392, 346
22	561	—

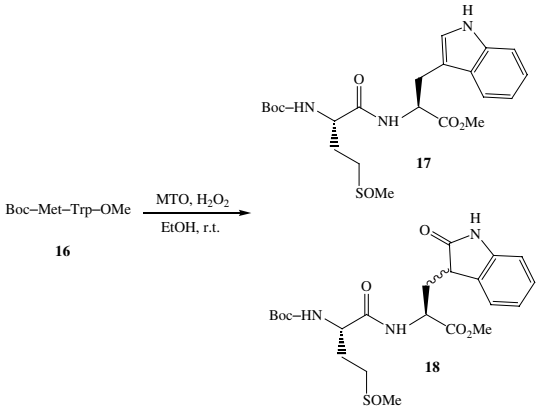
[a] ESI/MS tandem mass spectrometry analyses were performed by means of a TSQ Quantum Ultra AM ThermoFinnigan instrument.
[b] MS/MS analyses were performed only when necessary for a clear identification of products.



Scheme 4.



Scheme 5.

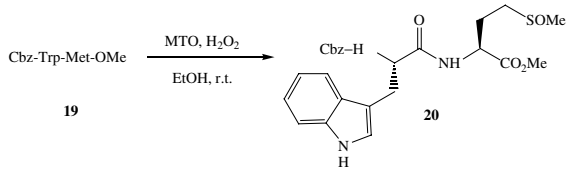


Scheme 6.

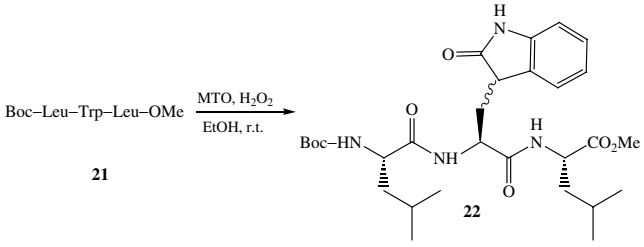
The oxidation of the dipeptide Boc-Leu-Trp-OMe **12** gave the selective functionalization of the Trp residue, compound **13** being recovered as a 4:1 mixture of diastereomers in 70% total yield (Scheme 5). Treatment of Cbz-Trp-Leu-OMe **14** under similar experimental conditions afforded the oxo-indole derivative **15** as the only isolated product in 69% yield (Scheme 5) showing again the absence of position selectivity.

Our attention was next addressed to study the behavior of Boc-Met-Trp-OMe **16** in order to evaluate the selectivity in the oxidation of dipeptides bearing two reactive amino acids. The oxidation of **16** gave the sulfoxide **17** and product **18**, in which both the sulfur and indole moieties are oxidized, in ca. 1:1 ratio (47% and 53% yield, respectively; Scheme 6). Sulfoxide **17** became the main reaction product (80%) along with low amount of **18** (10%) using 1.0 equiv of oxidant.

On the basis of these data, the Met residue appears to be more reactive than Trp toward MTO. Moreover, it is interesting to note that the oxidation of the sulfur moiety to sulfone, previously shown for Met, was not an operative process. Probably, after the oxidation of Met residue to sulfoxide, steric effects against the next approach of MTO peroxo complexes to sulfur atom can be operative. The possibility that the indole moiety become more reactive than the newly formed sulfoxide resi- due cannot be completely ruled out. Accordingly,



Scheme 7.



Scheme 8.

a quantitative conversion of substrate and quantitative yield of sulfoxide **20** were obtained in the oxidation of Cbz-Trp-Met-OMe **19** with 1.0 equiv of oxidant (Scheme 7).

Finally, the oxidation of tripeptide Boc-Leu-Trp-Leu-OMe **21** was studied. In this case, the Trp side-chain modified peptide **22** was selectively obtained in 75% yield (Scheme 8).

In conclusion, because of the high selectivity obtained in the side-chain oxidation of amino acids, one may suggest that MTO might readily interact with a number of proteins and biologically important peptides in a sequence specific manner.

Acknowledgements

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