

# Oxidation of Peptides by Methyl(trifluoromethyl)dioxirane: The Protecting Group Matters

Maria Rosaria Rella and Paul G. Williard\*

Department of Chemistry, Brown University, Providence, Rhode Island 02912

Paul\_Williard@Brown.edu

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t-Bu-O-C-N-PEPTIDE-C-O-CH<sub>3</sub>  $\longrightarrow$  N-Boc Oxidation  $F_3C_O$  $H_3C-C-N-PEPTIDE-C-O-CH_3 \longrightarrow$  Side Chain Oxidation

Representative Boc-protected and acetyl-protected peptide methyl esters bearing alkyl side chains undergo effective oxidation using methyl(trifluoromethyl)dioxirane (1b) under mild conditions. We observe a protecting group dependency in the chemoselectivity displayed by the dioxirane 1b. N-Hydroxylation occurs in the case of the Boc-protected peptides, and side chain hydroxylation takes place in the case of acetyl-protected peptides. Both are attractive transformations since they yield derivatized peptides that serve as valuable synthons.

#### Introduction

Several proteins and peptides are human therapeutic agents, and considerable effort is directed at the discovery of new analogues for novel or improved treatment of diseases.<sup>1</sup> One powerful approach is to chemically alter existing protein and peptide drugs. Structural modifications of these molecules might improve the pharmacokinetic properties of bioactive peptides or even convert inactive peptides to new drugs. In this paper we describe some useful transformations of peptides by dioxiranes with the goal of creating new drugs.

It is well-established that dimethyldioxirane (1a)  $(DMD)^2$  and methyl(trifluoromethyl)dioxirane (1b)  $(TFD)^3$  are effective reagents to carry out a variety of synthetically useful oxidations under mild conditions. Exemplary transformations are the *syn*stereoselective epoxidation of alkenes, oxidation of molecules containing atoms bearing lone pairs such as sulfides, sulfoxides, and amines, and O-insertion into C–H bonds of various functional groups such as aldehydes, secondary or primary alcohols, acyclic and cyclic ethers, and the Si–H bond of silanes.<sup>4</sup> Among these reactions is the remarkable O-insertion into "unactivated" C–H bonds of alkanes.<sup>5</sup> This reaction mimics that catalyzed by P450 enzymes.<sup>6</sup> The high reactivity and high selectivity displayed by dioxiranes, coupled with the mild reaction conditions (typically 0 °C to room temperature, neutral pH) and simple workup (removal of the solvent), encourage their use as the reagents of choice to carry out oxidation of natural products.<sup>5</sup> Additionally these reagents are very effective for oxidation of substrates sensitive to acidic or basic pH values.<sup>7</sup>

$$\begin{array}{c} \mathsf{R} & \mathsf{O} \\ \mathsf{H}_3\mathsf{C} & \mathsf{Ia} \ \mathsf{R} = \mathsf{C}\mathsf{H}_3 \\ \mathsf{H}_3\mathsf{C} & \mathsf{Ib} \ \mathsf{R} = \mathsf{C}\mathsf{F}_3 \end{array}$$

It has been reported that DMD selectively hydroxylates the alkyl side chains of *N*-Boc-protected  $\alpha$ -amino acid esters.<sup>8</sup> The results vary depending on the structure of the amino acid. These reactions are rather sluggish, requiring long reaction times (several days) for sizable substrate conversion. High regiose-lectivity for the O-insertion into the  $\gamma$ -CH bond of leucine (Leu)

<sup>\*</sup> Corresponding author.

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rather than into the  $\alpha$ -CH bond has been reported.<sup>8,9</sup> Thus, Boc-Leu-OMe has been found to yield the corresponding 4,4dimethyl-4-butanolide derivative. The latter is believed to be formed by selective O-insertion into the tertiary  $\gamma$ -CH bond of Leu followed by cyclization (eq 1).

$$\begin{array}{c} \overset{H}{\underset{H_{3}C}{}} \circ \overset{O}{\underset{CH_{3}}{}} & \overset{DMD \ (6 \ equiv)}{\underset{5 \ d, \ conv \ 45\%, \ yield \ 93\% \ (^{9})}{\underset{5 \ d, \ conv \ 33\%, \ yield \ 15\% \ (^{10})}{} \\ \end{array} \right) \\ & \overbrace{H_{3}C \ CH_{3}}{} \overset{H}{\underset{5 \ d, \ conv \ 33\%, \ yield \ 15\% \ (^{10})}{} \\ \end{array}$$

"Position selectivity" in the oxidation of peptides containing more than one Leu residue was also reported.<sup>8</sup>

Curci reported that oxidation of Boc-Leu-OMe with TFD (5 equiv) occurs more rapidly (6 h, 91% conversion) than with DMD, but it gives different selectivity. The major product for the oxidation with TFD is the *N*-hydroxy derivative of Boc-Leu-OMe (57% yield, no loss of enantiomeric excess) along with the *N*-hydroxy derivative of the butanolide (21% yield).<sup>10</sup> We have extended the study of the reaction of TFD with Boc-protected di- and tripeptide esters bearing alkyl side chains. In all the cases analyzed, we observe selective hydroxylation of the terminal nitrogen atom in short reaction times and remarkably mild reaction conditions. Our results offer a novel opportunity to directly hydroxylate linear peptides at the N-terminal position and an easy access to hydroxamic acids.<sup>11</sup>

These studies are important because hydroxamic acids have several biological activities. For example, they are inhibitors of secretases involved in Alzheimer's disease and blood pressure regulation,<sup>12</sup> chemotherapeutic agents,<sup>13</sup> anti-inflammatory agents for the treatment of asthma and rheumatoid arthritis,<sup>14</sup> antimalarial agents,<sup>15</sup> iron chelators,<sup>16</sup> and siderophores.<sup>11b</sup> Furthermore it has recently been shown that *N*-hydroxy peptides react readily with  $\alpha$ -keto acids to form amides.<sup>17</sup> Thus, the results reported herein yield synthons that can be utilized in this novel chemoselective amide ligation for polypeptide synthesis.<sup>17</sup>

In order to test whether the interesting N-terminal hydroxylation of peptides by TFD is unique for the Boc protection or

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whether it is a rather general phenomenon with N-protected peptides, we decided to explore the reactivity of some *N*-acetyl-protected amino acid and dipeptide esters bearing alkyl side chains. Hence, we observe high regioselective side chain hydroxylation in the reaction with *N*-acetyl-protected amino acids and dipeptides. We do not observe any oxidation of the *N*-acetyl moiety of these compounds. These observations provide easy access to side chain modifications of linear peptides containing only amidic nitrogen atoms, and of cyclic polypeptides and depsipeptides.<sup>18</sup>

## **Results and Discussion**

The results of the oxidation of the selected Boc-protected di- and tripeptide methyl esters with methyl(trifluoromethyl)-dioxirane are collected in Table 1.

Oxidation of *N*-Boc-Ala-Ala-OMe (**2**) with 2.4 equiv of TFD produced the Boc-*N*-hydroxy derivative **2a** in 78% isolated yield as the only product after 4 h of reaction at 0 °C in CH<sub>2</sub>Cl<sub>2</sub> (entry 1). The reaction is assumed to occur without loss of enantiomeric excess.<sup>10</sup> The structure of the compound **2a** and of the following oxidation products that are described in this work were unambiguously characterized by the combination of high-resolution mass and 1D and 2D NMR techniques. The reaction conditions adopted in the oxidation of dipeptide **2** were used in the oxidation of the other Boc-protected peptides bearing alkyl side chains.

Hydroxylation of the Boc-protected nitrogen was observed also in the case of *N*-Boc-Val-Ala-OMe (**3**) and *N*-Boc-Val-Ala-Ala-OMe (**4**) (entries 2–3). N(OH)-Boc-Val-Ala-OMe (**3a**) and N(OH)-Boc-Val-Ala-Ala-OMe (**4a**) were produced in 81% and 71% isolated yields, respectively, suggesting that hydroxylation of the Boc-protected nitrogen in peptides by a moderate excess of TFD is a generally useful reaction, regardless of the length of the peptides. Boc-Ala-Leu-OMe (**5**) was converted to N(OH)-Boc-Ala-Leu-OMe (**5a**) in a slightly lower yield (63% isolated yield) (entry 4).

The composition of products obtained in the oxidation of N-Boc-Leu-Ala-OMe (6) with 2.4 equiv of TFD was more complex (entry 5). N-Boc-Leu(y-OH)-Ala-OMe (6b) and N(OH)-Boc-Leu( $\gamma$ -OH)-Ala-OMe (**6c**) were isolated in 11% and 15% yield, respectively, in addition to the production of N(OH)-Boc-Leu-Ala-OMe (6a) as the major product in 46% isolated yield. In order to establish whether 6b and, in particular, the overoxidation product 6c were produced by the excess of dioxirane, oxidation of N-Boc-Leu-Ala-OMe with 1.2 equiv of TFD was investigated. Under these conditions, a similar distribution of products was obtained as when the reaction was run with 2.4 equiv of TFD (6a, 6b, and 6c were obtained in 25%, 13%, and 5% isolated yield, respectively). This result suggests that in the oxidation of N-Boc-Leu-Ala-OMe with TFD, O-insertion into the tertiary  $\gamma$ -CH bond kinetically competes with the oxidation of Boc-protected nitrogen, yet the N-Boc hydroxylation is the favored process. These results are in agreement with the outcome of the reaction of TFD with Boc-protected amino acid methyl esters bearing an alkyl side chain.<sup>10</sup> Hence, the terminal N-hydroxy peptide methyl esters can be obtained by facile and quantitative removal of the Boc protecting group using TFA.<sup>10</sup>

To the best of our knowledge, the work presented here represents the first example of direct transformation of carbamic-

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TABLE 1. Oxidation of Boc-Protected Di- and Tripeptide Methyl Esters 2-6 by Methyl(trifluoromethyl)dioxirane

Entry	Substrate	Ox/Sub <sup>a</sup>	Products	Isol. Yield
1	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	2.4	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	78%
2	$ \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c}$	2.4	$ \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c}$	81%
3	$\begin{array}{c} H_{3}C & O \\ H_{3}C & H_{3}C \\ H_{3}C & H_{3}C \\ H_{3}C & H_{3}C \\ H_{3}C & H_{3}C \\ H_{3}C & H_{3} \\ H_{3}C \\ H_$	2.4	$\begin{array}{c} H_{3}C & O \\ O \\ H_{3}C & O \\ O \\ O \\ H \\ O \\ H \\ O \\ H \\ O \\ CH_{3} \\ H \\ O \\ CH_{$	71%
4	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	2.4	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	63%
	, CH₃		$H_{3}C \xrightarrow{CH_{3}} H \xrightarrow{O} CH_{3}$ $H_{3}C \xrightarrow{O} H \xrightarrow{O} CH_{3}$ $H_{3}C \xrightarrow{O} CH_{3}$ $H_{3}C \xrightarrow{O} CH_{3}$ $Ga$	46% (25%)
5		2.4 (1.2)	$\begin{array}{c} H_{3}C \longrightarrow CH_{3} \\ H_{3}C \longrightarrow N \longrightarrow O \\ H_{3}C \longrightarrow N \longrightarrow O \\ H_{3}C \longrightarrow N \longrightarrow O \\ H \longrightarrow O \\ H \longrightarrow O \\ \mathbf{6b} \end{array}$	11% (13%)
			$ \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c}$	15% (5%)

The reactions were routinely run at 0 °C, for 4 h; the solvent composition was CH<sub>2</sub>Cl<sub>2</sub>/1,1,1-trifluoropropanone (TFP). <sup>*a*</sup> Molar ratio of dioxirane oxidant to substrate.

protected peptides into the terminal *N*-hydroxy derivatives in good yields and offers a valid alternative to the previous examples of synthesis of these compounds that require multistep procedures. The typical procedures for the preparation of terminal *N*-hydroxy peptides are coupling of an activated protected *N*-hydroxy amino acid with a peptide,<sup>11a</sup> coupling of an activated  $\alpha$ -oximino acid with a peptide followed by reduction and separation of the diastereomers,<sup>11a</sup> and synthesis of the oxime from a suitable  $\alpha$ -keto amide followed by reduction and separation of the diastereomers.<sup>11c</sup> The easy access to the terminal *N*-hydroxy peptides is likely to have a positive synthetic impact because these compounds are valuable synthons in a novel chemoselective amide ligation for the polypeptide synthesis by coupling with a peptide ketoacid,<sup>17</sup> and in the formation of hydroxamates by reaction with an acyl chloride.<sup>11c</sup> *N*-Hydroxy peptides are biologically interesting compounds on their own because they are involved in metabolic transformations, as demonstrated by their occurrence in animal and human tumors.<sup>11a,19</sup> Since they exhibit iron complexation properties, they have been adopted as antibacterial and antifungal agents and have been explored in the treatment of tumors.<sup>11b,13</sup>

In order to test whether the interesting N-terminal hydroxylation of peptides by TFD is unique for the Boc protection or it is a rather general phenomenon with N-protected peptides, we investigated the reactivity of some *N*-acetyl-protected amino acid and dipeptide esters, bearing alkyl side chains, toward TFD. We adopted the same conditions utilized for the oxidation of

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<b>ΓABLE 2.</b>	<b>Oxidation of Acetyl-Protected</b>	Amino Acid and Dipeptide	• Methyl Esters 7	7-13 by Methyl(triflue	oromethyl)dioxiran
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Entry	Substrate	Ox/Sub <sup>a</sup>	$T(h)^{b}$	Products	Isol. Yield
1	H <sub>3</sub> C-V <sup>CH<sub>3</sub></sup> H <sub>3</sub> C <sup>N</sup> N <sup>O</sup> CH <sub>3</sub> H <sub>3</sub> C <sup>N</sup> N <sup>O</sup> CH <sub>3</sub> H <sup>O</sup> O <sup>CH<sub>3</sub></sup>	2.4 (5.0)	5 (6)	$H_{3}C$ $H$	48% (82%)
2	H <sub>3</sub> C, B, CH <sub>3</sub> H <sub>3</sub> C, N, T, O, CH <sub>3</sub> H B <b>8</b>	2.4 (5.0)	5 (7)	H <sub>3</sub> C OH O CH <sub>3</sub> H <sub>3</sub> C N O CH <sub>3</sub> H O CH <sub>3</sub> <b>8a</b>	14% (44%)
3	$ \begin{array}{cccccc} H_{3}C & \downarrow CH_{3} \\ O & H & O \\ H_{3}C & N & \downarrow O \\ H & O & CH_{3} \end{array} $	2.4 (5.0)	5 (6)	$\begin{array}{c} \begin{array}{c} OH \\ H_{3}C & H \\ O \\ H_{3}C & H \\ H_{3}C & H \\ H \\ H \\ \end{array} \begin{array}{c} OH \\ H $	54% (73%)
4	$\begin{array}{c} O & CH_3 H & O \\ H_3C & N & H & O \\ H & O & CH_3 \\ H & O & CH_3 \\ 10 & CH_3 \end{array}$	2.4	5	$ \begin{array}{c}                                     $	36%
5	$H_{3C} \xrightarrow{\beta} CH_{3} O \xrightarrow{\beta} H_{3} O \xrightarrow{\beta} H_{3} O \xrightarrow{\beta} H_{3} O \xrightarrow{CH_{3}} O \xrightarrow{CH_{3}} O \xrightarrow{CH_{3}} O \xrightarrow{CH_{3}} O \xrightarrow{CH_{3}} O \xrightarrow{H_{3}} O \xrightarrow{CH_{3}} O \xrightarrow{H_{3}} O \xrightarrow{CH_{3}} O \xrightarrow{H_{3}} O \xrightarrow{H_{3}} O \xrightarrow{CH_{3}} O \xrightarrow{H_{3}} O \xrightarrow{H_{3}$	2.4 (5.0)	5 (7)	$H_{3}C \xrightarrow{OH} CH_{3} H_{3}O \xrightarrow{H} OH CH_{3} H_{3}O \xrightarrow{H} OH CH_{3} H_{3}O \xrightarrow{H} O O \oplus{H} O O \xrightarrow{H} O O$	12% (29%)
6	$H_{3}C \xrightarrow{\gamma}CH_{3}$ $H_{3}C \xrightarrow{\gamma}N$	2.4 (5.0)	5 (6)	$H_{3C} \xrightarrow{CH_{3}} H \xrightarrow{O} H \xrightarrow{H} O$ $H_{3C} \xrightarrow{H} N \xrightarrow{H} O \xrightarrow{CH_{3}} O \xrightarrow{CH_{3}} H \xrightarrow{O} O$ $H_{3C} \xrightarrow{H} O \xrightarrow{CH_{3}} O \xrightarrow{CH_{3}} O \xrightarrow{CH_{3}} O$ $H_{3C} \xrightarrow{H} O \xrightarrow{CH_{3}} O \xrightarrow{C} O \xrightarrow{CH_{3}} O \xrightarrow{CH_{3}} O \xrightarrow{CH_{3}} O \xrightarrow{CH_{3}} O \xrightarrow{CH_{3}} O \xrightarrow{CH_{3}} O \xrightarrow{C} O \xrightarrow{CH_{3}} O \xrightarrow{C} O \xrightarrow{CH_{3}} O \xrightarrow{CH_{3}} O \xrightarrow{CH_{3}} O \xrightarrow{CH_{3}} O \xrightarrow{C} O \xrightarrow{CH_{3}} O \xrightarrow{CH_{{3}}} O \xrightarrow{CH_{{3}}} O \xrightarrow{CH_{{3}}} O \xrightarrow{CH_{{3}} O \xrightarrow{CH_{{3}}} O $	48% (60%)
7	$\begin{array}{c} H_{3}C \xrightarrow{CH_{3}} O \xrightarrow{H_{3}} O O H$	2.4	5	$H_{3C} \xrightarrow{H_{3}C} H_{3} \xrightarrow{H_{3}} \xrightarrow{H_{3}} H_{3} $	36%

The reactions were routinely run at 0 °C; the solvent composition was acetone/1,1,1-trifluoropropanone (TFP). <sup>*a*</sup> Molar ratio of dioxirane oxidant to substrate. <sup>*b*</sup> Reaction time.

the Boc-protected peptide methyl esters in the oxidation of acetylamino acid and dipeptide methyl esters (2.4 equiv, 0 °C) for comparative reasons. Acetone was the cosolvent along with 1,1,1-trifluoroacetone in these reactions, rather than methylene chloride, because some valine-containing substrates were more soluble in acetone. Longer reaction times (5 h) were necessary for full consumption of the oxidant. The results of the oxidation of the selected acetyl-protected amino acid and dipeptide methyl esters with methyl(trifluoromethyl)dioxirane are collected in Table 2.

Under these conditions Ac-Leu-OMe (7) was converted to the 4,4-dimethyl-4-butanolide derivative (7a) in 48% isolated yield by reaction with 2.4 equiv of TFD (entry 1). Compound 7a is formed by O-insertion into the tertiary  $\gamma$ -CH bond of leucine, followed by cyclization. Ac-Val-OMe (8) underwent oxidation at the tertiary  $\beta$ -CH bond of value in 14% isolated yield (entry 2). The lower yield measured in the case of the reaction of Ac-Val-OMe with TFD with respect to 7 can be rationalized by considering the electronic deactivation, because the amidic functionality is closer to the  $\beta$ -CH bond of valine, relative to the  $\gamma$  position of leucine. This rationale holds for the observed behavior of dimethyldioxirane in the reaction with Boc-Leu-OMe and Boc-Val-OMe, where it is reported that with Boc-Leu-OMe, the 4,4-dimethyl-4-butanolide derivative was produced, whereas Boc-Val-OMe did not react with DMD.<sup>8</sup>

The oxidation products of *N*-Ac-Leu-Ala-OMe (**9**) and *N*-Ac-Ala-Leu-OMe (**10**) were *N*-Ac-Leu( $\gamma$ -OH)-Ala-OMe (**9a**) (54% isolated yield) and *N*-Ac-Ala-2-amino-4,4-dimethyl-4-butanolide (**10a**) (36% isolated yield), respectively (entries 3 and 4). The  $\beta$ -CH bond of value in Ac-Val-Ala-OMe (**11**) reacted to a lower extent, as in the simpler case **8**, *N*-Ac-Val( $\beta$ -OH)-Ala-OMe (**11a**) being produced in 12% yield (entry 5). *N*-Ac-Leu-Val-

We observed a trend in the oxidation of the two couples of dipeptides containing leucine: when the leucine is the Cterminal residue (cf., N-Ac-Ala-Leu-OMe and N-Ac-Val-Leu-OMe), the O-insertion into the tertiary  $\gamma$ -CH occurs to a lower extent than in the case when leucine is the N-teminal residue (cf., N-Ac-Leu-Ala-OMe and N-Ac-Leu-Val-OMe). This trend can be rationalized considering that dioxiranes are sensitive to the surrounding stereoelectronic environment of the reactive site.<sup>4</sup> NOESY NMR experiments in acetone- $d_6$  at 0 °C were carried out on one of the two couples of peptides incorporating N-terminal leucine and C-terminal leucine respectively, 12 and 13. The choice of the solvent and the temperature in the 2D NOE experiments reflected the conditions adopted during the reactions of N-Ac-Leu-Val-OMe and N-Ac-Val-Leu-OMe with TFD. The aim of these NMR investigations was to determine whether structural features of the substrates in solution might facilitate (in the case of 12) and/or obstruct (in the case of 13) the approach of the oxidant to the tertiary  $\gamma$ -CH bond of the leucine fragment. NOESY spectra are presented in the Supporting Information, supporting this explanation.

NOE experiments reveal the presence of a more dense network of NOE effects involving the  $\gamma$ -H atom of the leucine fragment in the case of *N*-Ac-Val-Leu-OMe in respect to *N*-Ac-Leu-Val-OMe. This observation suggests a more crowded environment surrounding the reactive site of leucine, when leucine is not the N-terminal residue. This steric factor might account for the lower reactivity exhibited by the tertiary  $\gamma$ -CH bond of the leucine in *N*-Ac-Val-Leu-OMe with respect to *N*-Ac-Leu-Val-OMe.

As a complement to the NMR studies, molecular mechanics calculations (MMX, PCMODEL) were carried out on **12** and **13**, without using any conformational constraints. The lower energy conformations of *N*-Ac-Leu-Val-OMe and *N*-Ac-Val-Leu-OMe (see the Supporting Information) are in agreement with the 2D NOE correlations for the  $\gamma$ -H atom of the leucine obtained experimentally and show the presence of one intramolecular hydrogen bond in both compounds. The specific intramolecular hydrogen bonds in the structures of *N*-Ac-Leu-Val-OMe and *N*-Ac-Val-Leu-OMe predicted by the MMX calculations are in agreement with acid-catalyzed hydrogen/deuterium (H/D) exchange experiments,<sup>20</sup> carried out at 0 °C by shaking the solution of compounds **12** and **13** in acetone-*d*<sub>6</sub> with solution of TFA in D<sub>2</sub>O (for the experimental details, see the Supporting Information).

We attempted to improve the yields of the synthetically more promising substrates (7, 8, 9, 11, 12) by using 5 equiv of dioxirane 1b. Ac-Leu-OMe (7) was oxidized to 7a in 82% isolated yield in 6 h of reaction. Ac-Val-OMe (8) and Ac-Val-Ala-OMe (11) were converted to 8a and 11a in 44% and 29% isolated yield, respectively, in 7 h of reaction. *N*-Ac-Leu-Ala-OMe (9) and *N*-Ac-Leu-Val-OMe (12) underwent oxidation in 6 h producing 9a and 12a in 73% and 60% isolated yield, respectively. In general TFD oxidizes the acetyl-protected amino acid and dipeptide methyl esters 7-13 exclusively at the tertiary CH bond of the aliphatic side chains, leaving the terminal protected nitrogen unaffected.

It is well-established that dioxiranes **1a**,**b** are able to oxidize amines and *N*-heteroarenes. The mechanism of the oxidation of the nitrogen atom is still controversial. Ab initio calculations<sup>21</sup> indicate that the O-transfer of DMD to primary amines has purely electrophilic character, and experimental evidence<sup>22</sup> supports an S<sub>N</sub>2 mechanism in the nitrogen lone pair oxidation of *N*-heteroarenes by **1a**. Dimethyldioxirane is incapable to transfer oxygen to the nitrogen of unconstrained carbamates and amides. Our observations attest to the fact that methyl(trifluoromethyl)dioxirane is able to hydroxylate secondary carbamic nitrogen atoms but not the unconstrained amidic nitrogen atoms.

Although carbamates are structurally similar to amides, they present different physicochemical features, due to the steric and electronic perturbations introduced by the additional oxygen of the carbamates. NMR studies and theoretical calculations<sup>23</sup> reveal that the barriers to rotation in carbamates are typically 3–4 kcal/mol lower than in structurally related amides (~20 kcal/mol). The effect of the additional oxygen in carbamates, eventually as competitor of the nitrogen in conjugation with the carbonyl, can be seen in the solid state as well. A nonplanar distortion ( $\pm$  9° to 11°)<sup>24</sup> of the amidic moiety of carbamates is appreciably higher than the torsion angle  $\omega$  typically found in the amidic bonds of peptides. Accordingly, the amide C–N bond of carbamates is ~0.03 Å longer than a C–N bond on the amide in a planar arrangement (~1.32 Å).<sup>24a</sup>

If we propose an  $S_N 2$  mechanism<sup>22</sup> for the nitrogen lone pair of carbamate oxidation by the dioxirane TFD, the higher conformational flexibility exerted by the carbamate functionality with respect to the amidic functionality can be accounted for by the ability of TFD to transfer oxygen to the lone pair of the carbamic, but not of the amidic, nitrogen. It is reasonable to assume that an increase in conformational flexibility might have as a consequence an increase in the nucleophilic character of the lone pair of nitrogen of the N-terminal residue in the Bocprotected peptides in respect to the acetyl-protected peptides.

### Conclusions

The chemoselectivity of TFD in the oxidation of protected peptides is dictated by the protecting group of the N-terminal residue of the peptides. Hydroxylation of the terminal nitrogen atom in Boc-protected di- and tripeptide methyl esters bearing alkyl side chains can be obtained in good yields by oxidation of the corresponding Boc-protected peptides with methyl-(trifluoromethyl)dioxirane in short reaction times and with remarkably mild reaction conditions. This is a synthetically useful transformation since *N*-hydroxy peptides are important synthons in the preparation of biologically active molecules.<sup>11,17</sup>

*N*-Acetyl-protected amino acid and dipeptide methyl esters bearing alkyl side chains undergo exclusively high regioselective side chain hydroxylation when they react with TFD. The tertiary  $\gamma$ -CH bond of the leucine residue displays higher reactivity in comparison to the tertiary  $\beta$ -CH bond of the valine residue. Furthermore, if leucine is one of the two components of *N*-acetyl

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dipeptide methyl esters, the  $\gamma$  position of leucine is hydroxylated more effectively when it is the N-terminal residue of the dipeptides.

These findings provide easy access to side chain modifications of linear peptides containing only amidic nitrogen atoms and of cyclic peptides and depsipeptides, leaving intact the backbone structure. We are currently working on selective side chain modifications by TFD of cyclic peptides of biological relevance.

## **Experimental Section**

Acetyl-protected amino acid methyl esters were obtained from the corresponding amino acids. *tert*-Butoxycarbonyl and acetyl diand tripeptide methyl esters were prepared by protecting the commercially available dipeptides or following coupling procedures in solution.<sup>25</sup> Solutions of TFD **1b** (0.4–0.5 N) in 1,1,1-trifluoroacetone were isolated according to the procedure described by Curci.<sup>3</sup> The procedure described for the oxidation of **2** is representative for oxidations of substrates **3–6** using 2.4 equiv of TFD, unless differently specified.

N-Hydroxy-N-tert-butoxycarbonyl-alanylalanine methyl ester (2a): A standardized solution of TFD (1b) in 1,1,1-trifluoropropanone (TFP) (0.86 mL, 0.4 M,  $3.4 \times 10^{-4}$  mol) was added in one portion to a stirred solution of N-Boc-Ala-Ala-OMe (2) (0.0393,  $1.43 \times 10^{-4}$  mol) in CH<sub>2</sub>Cl<sub>2</sub> (0.5 mL) at 0 °C. The progress of the reaction was monitored by TLC (1:1:1 hexanes/Et<sub>2</sub>O/acetone; detection phosphomolybdic acid, PMA). After 4 h of reaction time, the solvent was removed by rotary evaporation. N-Hydroxy-N-tertbutoxycarbonyl-alanylalanine methyl ester (2a) (0.0326 g,  $1.12 \times$  $10^{-4}$  mol) was isolated by flash chromatography (1:1:1 hexanes/ Et<sub>2</sub>O/acetone) in 78.3% isolated yield as white solid; 78.3% yield based on the recovered starting material; 95.0% substrate conversion; <sup>1</sup>H NMR (acetone- $d_6$ , 400 MHz)  $\delta$  8.12 (s, 1 H, Ala<sup>1</sup>-NOH), 7.32 (m, 1 H, Ala<sup>2</sup>-NH), 4.63 (q, 1 H, J = 7.1 Hz, Ala<sup>1</sup>-C<sup> $\alpha$ </sup>H), 4.46 (p, 1 H, J = 7.3 Hz, Ala<sup>2</sup>-C<sup> $\alpha$ </sup>H), 3.68 (s, 3 H, OCH<sub>3</sub>), 1.45 (s, 9 H, Boc-CH<sub>3</sub>), 1.37 (m, 6 H, Ala<sup>1,2</sup>-CH<sub>3</sub>); <sup>13</sup>C NMR (acetone-d<sub>6</sub>, 75 MHz) δ 174.7 (C), 172.8 (C), 158.8 (C), 82.6 (Boc-C), 59.7 (Ala<sup>1</sup>-C<sup>α</sup>H), 53.4 (OCH<sub>3</sub>), 49.7 (Ala<sup>2</sup>-C<sup>α</sup>H), 29.2 (Boc-CH<sub>3</sub>), 18.9 and 14.9 (Ala<sup>1,2</sup>-CH<sub>3</sub>); HRMS-FAB (M + Na<sup>+</sup>) calculated for C<sub>12</sub>H<sub>22</sub>NaN<sub>2</sub>O<sub>6</sub> 313.1376, found 313.1384.

*N*-Hydroxy-*N*-*tert*-butoxycarbonyl-valinylalanine methyl ester (**3a**): 86.0% substrate conversion, 93.7% yield based on the recovered starting material, 80.6% isolated yield; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 7.82 (s, 1 H, Val-NOH), 6.94 (d, 1 H, *J* = 7.3 Hz, Ala-N*H*), 4.56 (p, 1 H, *J* = 7.2 Hz, Ala-C<sup>α</sup>*H*), 4.14 (d, 1 H, *J* = 9.6 Hz, Val-C<sup>α</sup>*H*), 3.72 (s, 3 H, OCH<sub>3</sub>), 2.36 (m, 1 H, *J* = 9.5 Hz, *J'* = 6.7 Hz, Val-C<sup>β</sup>*H*), 1.46 (s, 9 H, Boc-C*H*<sub>3</sub>), 1.40 (d, 3 H, *J* = 7.2 Hz, Ala-C<sup>α</sup>H), 51.7 (*C*), 171.0 (*C*), 157.0 (*C*), 82.5 (Boc-C), 67.8 (Val-C<sup>α</sup>H), 52.4 (OCH<sub>3</sub>), 47.9 (Ala-C<sup>α</sup>H), 28.21 and 28.18 (Val-C<sup>β</sup>H and Boc-CH<sub>3</sub>), 19.6 and 19.2 (Val-C<sup>α</sup>/H<sub>3</sub>), 18.0 (Ala-CH<sub>3</sub>); HRMS-FAB (M + Na<sup>+</sup>) calculated for C<sub>14</sub>H<sub>26</sub>NaN<sub>2</sub>O<sub>6</sub> 341.1689, found 341.1695.

*N*-Hydroxy-*N*-tert-butoxycarbonyl-valinylalanylalanine methyl ester (4a): 77.5% substrate conversion, 91.4% yield based on the recovered starting material, 70.8% isolated yield; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  7.31 (s, 1 H, Val-NOH), 6.84 (d, 1 H, J = 7.4 Hz, Ala<sup>2.3</sup>-NH), 6.73 (d, 1 H, J = 7.3 Hz, Ala<sup>3.2</sup>-NH), 4.55 (p, 1 H, J = 7.3 Hz, Ala<sup>3.2</sup>-C<sup>\alpha</sup>H), 4.52 (p, 1 H, J = 7.2 Hz, Ala<sup>2,3</sup>-C<sup> $\alpha$ </sup>H), 4.15 (d, 1 H, J = 9.6 Hz, Val-C<sup> $\alpha$ </sup>H), 3.74 (s, 3 H, OCH<sub>3</sub>), 2.38 (m, 1 H, Val-C<sup> $\beta$ </sup>H), 1.48 (s, 9 H, Boc-CH<sub>3</sub>), 1.40 (d, 6 H, J = 7.1 Hz, two Ala-CH<sub>3</sub>), 1.01 (m, 6 H, Val-C<sup> $\gamma$ , $\gamma'$ H<sub>3</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz)  $\delta$  173.1 (C), 171.4 (C), 171.3 (C), 156.7 (C), 82.5 (Boc-C), 67.8 (Val-C<sup> $\alpha$ </sup>H), 52.5 (OCH<sub>3</sub>), 48.7 (Ala<sup>2.3</sup>-C<sup> $\alpha$ </sup>H), 48.1 (Ala<sup>3.2</sup>-C<sup> $\alpha$ </sup>H), 28.25 (Val-C<sup> $\beta$ </sup>H), 28.22 (Boc-CH<sub>3</sub>), 19.5 and 19.4 (Val-C<sup> $\gamma$ , $\gamma'$ H<sub>3</sub>), 18.1 (two Ala-CH<sub>3</sub>); HRMS-FAB (M + Na<sup>+</sup>) calculated for C<sub>17</sub>H<sub>31</sub>NaN<sub>3</sub>O<sub>7</sub> 412.2060, found 412.2070.</sup></sup>

*N*-Hydroxy-*N*-tert-butoxycarbonyl-alanylleucine methyl ester (5a): 83.8% substrate conversion, 75.4% yield based on the recovered starting material, 63.2% isolated yield; <sup>1</sup>H NMR (acetoned<sub>6</sub>, 400 MHz) δ 8.07 (s, 1 H, Ala-NO*H*), 7.25 (m, 1 H, Leu-N*H*), 4.63 (p, 1 H, J = 7.1 Hz, Ala-C<sup>α</sup>*H*), 4.54 (m, 1 H, Leu-C<sup>α</sup>*H*), 3.68 (s, 3 H, OC*H*<sub>3</sub>), 1.78–1.50 (m, 3 H, Leu-C*H*<sub>2</sub> and Leu-C<sup>γ</sup>*H*), 1.45 (s, 9 H, Boc-C*H*<sub>3</sub>), 1.40 (d, 3 H, J = 7.1 Hz, Ala-C*H*<sub>3</sub>), 0.91 (m, 6 H, Leu-C<sup>δ,δ'</sup>H<sub>3</sub>); <sup>13</sup>C NMR (acetone-d<sub>6</sub>, 100 MHz) δ 174.6 (C), 173.3 (C), 158.8 (C), 82.6 (Boc-C), 59.8 (Ala-C<sup>α</sup>H), 53.3 (OCH<sub>3</sub>), 52.2 (Leu-C<sup>α</sup>H), 42.7 (Leu-CH<sub>2</sub>), 29.3 (Boc-CH<sub>3</sub>); 1RMS-FAB (M + Na<sup>+</sup>) calculated for C<sub>15</sub>H<sub>28</sub>NaN<sub>2</sub>O<sub>6</sub> 355.1845, found 355.1852.

N-Hydroxy-N-tert-butoxycarbonyl-ambo-leucyl-ambo-alanine methyl ester (6a): for the oxidation of 6 with 2.4 equiv of TFD: 85.4% substrate conversion, 53.8% yield based on the recovered starting material, 46.0% isolated yield; for the oxidation of 6 with 1.2 equiv of TFD: 50.4% substrate conversion, 49.3% yield based on the recovered starting material, 24.8% isolated yield; <sup>1</sup>H NMR (acetone- $d_6$ , 400 MHz)  $\delta$  8.08 (br s, 1 H, Leu-NOH), 7.41 (m, 1 H, Ala-NH), 4.65 (m, 1 H, Leu-C $^{\alpha}$ H), 4.45 (p, 1 H, J = 7.2 Hz, Ala-C<sup>α</sup>H), 3.68 (s, 3 H, OCH<sub>3</sub>), 1.92 and 1.63 (two m, 2 H, Leu-CH<sub>2</sub>), 1.68 (m, 1 H, Leu-C<sup> $\gamma$ </sup>H), 1.46 (s, 9 H, Boc-CH<sub>3</sub>), 1.35 (d, 3 H, J = 7.2 Hz, Ala-CH<sub>3</sub>), 0.93 (m, 6 H, Leu-C<sup> $\delta,\delta'$ </sup>H<sub>3</sub>); <sup>13</sup>C NMR (acetone-d<sub>6</sub>, 100 MHz) δ 174.6 (C), 173.3 (C), 158.7 (C), 82.4 (Boc-C), 62.0 (Leu- $C^{\alpha}$ H), 53.4 (OCH<sub>3</sub>), 49.7 (Ala- $C^{\alpha}$ H), 38.9 (Leu-CH<sub>2</sub>), 29.4 (Boc-CH<sub>3</sub>), 26.4 (Leu-C<sup>γ</sup>H), 24.7 and 22.5 (Leu- $C^{\delta,\delta'}$ H<sub>3</sub>), 18.9 (Ala-CH<sub>3</sub>); HRMS-FAB (M + Na<sup>+</sup>) calculated for C<sub>15</sub>H<sub>28</sub>NaN<sub>2</sub>O<sub>6</sub> 355.1845, found 355.1848.

*N-tert*-Butoxycarbonyl-ambo- $\gamma$ -hydroxyleucyl-ambo-alanine methyl ester (6b): for the oxidation of 6 with 2.4 equiv of TFD: 85.4% substrate conversion, 12.3% yield based on the recovered starting material, 10.5% isolated yield; for the oxidation of 6 with 1.2 equiv of TFD: 50.4% substrate conversion, 24.7% yield based on the recovered starting material, 12.5% isolated yield; <sup>1</sup>H NMR  $(CDCl_3, 400 \text{ MHz}) \delta$  7.03 and 6.98 (two br d, 1 H, J = 7.2 Hz, Ala-N*H*), 5.71 and 5.65 (two br d, 1 H, J = 6.5 Hz, Leu-N*H*), 4.59 (p, 1 H, J = 7.3 Hz, Ala-C<sup> $\alpha$ </sup>H), 4.29 (m, 1 H, Leu-C<sup> $\alpha$ </sup>H), 3.74 (s, 3 H, OCH<sub>3</sub>), 2.04 and 1.79 (two m, 2 H, Leu-CH<sub>2</sub>), 1.47 (s, 9 H, Boc-CH<sub>3</sub>), 1.43 and 1.42 (two d, 3 H, J = 7.2 Hz, Ala-CH<sub>3</sub>), 1.32 (m, 6 H, Leu- $C^{\delta,\delta'}H_3$ ); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz)  $\delta$  173.3 and 173.2 (C), 172.4 (C), 155.9 and 155.8 (C), 80.3 (Boc-C), 70.4 (Leu-C<sup> $\gamma$ </sup>OH), 52.5 and 52.4 (OCH<sub>3</sub>), 51.9 (Leu-C<sup> $\alpha$ </sup>H), 48.1 and 48.0 (Ala- $C^{\alpha}$ H), 44.9 (Leu-CH<sub>2</sub>), 29.7 (Leu- $C^{\delta,\delta'}$ H<sub>3</sub>), 28.3 (Boc- $CH_3$ ), 18.2 (Ala- $CH_3$ ); HRMS-FAB (M + Na<sup>+</sup>) calculated for C<sub>15</sub>H<sub>28</sub>NaN<sub>2</sub>O<sub>6</sub> 355.1845, found 355.1850.

*N*-Hydroxy-*N*-tert-butoxycarbonyl-ambo-γ-hydroxyleucylambo-alanine methyl ester (6c): for the oxidation of 6 with 2.4 equiv of TFD: 85.4% substrate conversion, 17.3% yield based on the recovered starting material, 14.8% isolated yield; for the oxidation of 6 with 1.2 equiv of TFD: 50.4% substrate conversion, 10.8% yield based on the recovered starting material, 5.4% isolated yield; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 7.61 (br s, 1 H, Leu-NOH), 7.12 (d, 1 H, J = 7.0 Hz, Ala-NH), 4.81 (m, 1 H, Leu-C<sup>a</sup>H), 4.57 (p, 1 H, J = 7.3 Hz, Ala-C<sup>a</sup>H), 3.75 (s, 3 H, OCH<sub>3</sub>), 2.14 (d, 2 H, J = 7.2 Hz, Leu-CH<sub>2</sub>), 1.50 (s, 9 H, Boc-CH<sub>3</sub>), 1.42 (d, 3 H, J =7.2 Hz, Ala-CH<sub>3</sub>), 1.34 and 1.33 (two br s, 6 H, Leu-C<sup>δ,δ'</sup>H<sub>3</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 173.2 and 173.0 (C), 171.7 and 171.5 (C), 156.4 and 156.3 (C), 82.6 and 82.5 (Boc-C), 70.4 (Leu-C<sup>γ</sup>-OH), 59.4 (Leu-C<sup>α</sup>H), 52.5 and 52.4 (OCH<sub>3</sub>), 48.1 (Ala-C<sup>α</sup>H), 40.0 (Leu-CH<sub>2</sub>), 31.2 and 31.0 (Leu-C<sup>δ,δ'</sup>H<sub>3</sub>), 28.3 and 28.2 (Boc-CH<sub>3</sub>),

<sup>(25)</sup> For a general procedure, see: (a) Garner, P.; Park, J. M. Org. Synth. **1992**, 70, 18–27. (b) Bodanszky, M.; Bodanszky, A. The Practice of Peptide Synthesis; Springer-Verlag: New York, 1984; pp 103–104. (c) Zielinski, T.; Achmatowicz, M.; Jurczak, J. Tetrahedron: Asymmetry **2002**, 13, 2053– 2059. (d) Bretschneiter, T.; Miltz, W.; Munster, P.; Steglich, W. Tetrahedron **1988**, 44, 5403–5414. (e) Reddy, A. V.; Ravindranath, B. Synth. Commun. **1992**, 22, 257–264. (f) Sprout, C. M; Seto, C. T. J. Org. Chem. **2003**, 68, 7788–7794.

18.1 (Ala-CH<sub>3</sub>); HRMS-FAB (M + Na<sup>+</sup>) calculated for  $C_{15}H_{28}$ -NaN<sub>2</sub>O<sub>7</sub> 371.1794, found 371.1780.

The procedure described for the oxidation of **7** is representative for oxidations of substrates 8-13 using 2.4 equiv of TFD, unless differently specified.

**2-(Acetylamino)-4,4-dimethyl-4-butanolide (7a):** A standardized solution of TFD (**1b**) in 1,1,1-trifluoropropanone (TFP) (3.50 mL, 0.4 M, 1.41  $\times$  10<sup>-3</sup> mol) was added in one portion to a stirred solution of Ac-Leu-OMe (**7**) (0.1099 g, 5.870  $\times$  10<sup>-4</sup> mol) in acetone (1 mL) at 0 °C. The reaction progress was monitored by TLC (1:1:1 hexanes/Et<sub>2</sub>O/acetone; detection PMA). After 5 h of reaction time, the solvent was removed by rotary evaporation. 2-(Acetylamino)-4,4-dimethyl-4-butanolide (**2a**) (0.0484 g, 2.83  $\times$  10<sup>-4</sup> mol) was isolated by flash chromatography (1:1:1 hexanes/ Et<sub>2</sub>O/acetone) in 48.2% yield as white solid.

**2-(Acetylamino)-4,4-dimethyl-4-butanolide (7a):** for the oxidation of **7** with 2.4 equiv of TFD: 48.2% isolated yield; for the oxidation of **7** with 5.0 equiv of TFD: 95.0% substrate conversion, 82.3% yield based on the recovered starting material, 82.3% isolated yield; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  7.15 (d, 1 H, *J* = 7.3 Hz, N*H*), 4.75 (m, 1 H, *CH*), 2.45 and 1.94 (two m, 2H, *CH*<sub>2</sub>), 1.93 (s, 3 H, C(O)*CH*<sub>3</sub>), 1.40 and 1.34 (two s, 6 H, two *CH*<sub>3</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz)  $\delta$  175.0 (*C*), 170.6 (*C*), 82.4 (*C*<sup>4</sup>), 49.7 (*CH*), 41.1 (*CH*<sub>2</sub>), 28.6 and 26.8 (two *CH*<sub>3</sub>), 22.5 (C(O)*CH*<sub>3</sub>); HRMS-FAB (M + Na<sup>+</sup>) calculated for C<sub>8</sub>H<sub>13</sub>NaNO<sub>3</sub> 194.0793, found 194.0796.

*N*-Acetyl-β-hydroxyvaline methyl ester (8a): for the oxidation of **8** with 2.4 equiv of TFD: 49.8% substrate conversion, 29.0% yield based on the recovered starting material, 14.4% isolated yield; for the oxidation of **8** with 5.0 equiv of TFD: 60.0% substrate conversion, 73.0% yield based on the recovered starting material, 43.8% isolated yield; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) δ 6.38 (m, 1 H, NH), 4.52 (d, 1 H, J = 8.8, C<sup>α</sup>H), 3.77 (s, 3 H, OCH<sub>3</sub>), 2.05 (s, 3 H, C(O)CH<sub>3</sub>), 1.26 and 1.25 (two s, 6 H, C<sup>γ,γ</sup>H<sub>3</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 172.2 (C), 170.4 (C), 71.8 (C<sup>β</sup>OH), 59.7 (C<sup>α</sup>H), 52.3 (OCH<sub>3</sub>), 26.8 and 26.5 (C<sup>γ,γ</sup>H<sub>3</sub>) 23.1 (C(O)CH<sub>3</sub>); HRMS-FAB (M + Na<sup>+</sup>) calculated for C<sub>8</sub>H<sub>15</sub>NaNO<sub>4</sub> 212.0899, found 212.0891.

N-Acetyl-ambo-y-hydroxyleucyl-ambo-alanine methyl ester (9a): for the oxidation of 9 with 2.4 equiv of TFD: 69.6% substrate conversion, 77.0% yield based on the recovered starting material, 53.6% isolated yield; for the oxidation of 9 with 5.0 equiv of TFD: 95.0% substrate conversion, 72.6% yield based on the recovered starting material, 72.6% isolated yield; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  7.41 (d, 1 H, J = 7.1 Hz, Ala-NH), 6.90 (m, 1 H, Leu-N*H*), 4.61 (m, 1 H, Leu-C<sup> $\alpha$ </sup>*H*), 4.50 (p, 1 H, *J* = 7.3 Hz, Ala- $C^{\alpha}H$ ), 3.73 and 3.72 (two s, 3 H, OCH<sub>3</sub>), 2.07 - 2.02 and 1.87 -1.76 (m, 2 H, Leu-CH<sub>2</sub>), 2.01 and 2.00 (two s, 3 H, C(O)CH<sub>3</sub>), 1.39 (d, 3 H, J = 7.2 Hz, Ala-CH<sub>3</sub>), 1.28 (m, 6 H, Leu-C<sup> $\delta,\delta'$ </sup>H<sub>3</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz) δ 173.3 and 173.2 (C), 172.1 and 172.0 (C), 170.8 and 170.5 (C), 70.2 (Leu-C<sup>v</sup>OH), 52.5 (OCH<sub>3</sub>), 50.6 and 50.4 (Leu- $C^{\alpha}$ H), 48.2 (Ala- $C^{\alpha}$ H), 45.1 and 44.4 (Leu-CH<sub>2</sub>), 30.3, 30.0, 29.9 and 29.6 (Leu-C<sup>ô, 6'</sup>H<sub>3</sub>), 23.2 (C(O)CH<sub>3</sub>), 17.8 (Ala-CH<sub>3</sub>); HRMS-FAB (M + Na<sup>+</sup>) calculated for  $C_{12}H_{22}NaN_2O_5$ 297.1426, found 297.1435.

**2-**(*N*-Acetyl-alanylamino)-4,4-dimethyl-4-butanolide (10a): 65.5% substrate conversion, 54.3% yield based on the recovered starting material, 35.6% isolated yield; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  7.37 (d, 1 H, *J* = 6.9 Hz, C<sup>2</sup>NH), 6.48 (d, 1 H, *J* = 7.3 Hz, Ala-NH), 4.67 (m, 1 H, C<sup>2</sup>H), 4.53 (p, 1 H, *J* = 7.1 Hz, Ala-C<sup>\alpha</sup>H), 2.54 and 2.11 (two m, 2 H, CH<sub>2</sub>), 2.00 (s, 3 H, C(O)CH<sub>3</sub>), 1.50 and 1.42 (two s, 6 H, two CH<sub>3</sub>) 1.38 (d, 3 H, *J* = 7.0 Hz, Ala-CH<sub>3</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz)  $\delta$  174.2 (*C*), 172.9 (*C*), 170.4 (*C*), 82.5 (*C*<sup>4</sup>), 50.3 (*C*<sup>2</sup>H), 48.6 (Ala- $C^{\alpha}$ H), 41.1 (*C*H<sub>2</sub>), 28.8 and 27.2 (two *C*H<sub>3</sub>), 23.1 (*C*(O)*C*H<sub>3</sub>), 18.1 (Ala-*C*H<sub>3</sub>); HRMS-FAB (M + Na<sup>+</sup>) calculated for C<sub>11</sub>H<sub>18</sub>NaN<sub>2</sub>O<sub>4</sub> 265.1164, found 265.1170.

*N*-acetyl-*ambo*- $\beta$ -hydroxyvalyl-*ambo*-alanine methyl ester (11a): For the oxidation of 11 with 2.4 equiv of TFD: 34.4% substrate conversion, 35.9% yield based on the recovered starting material, 12.3% isolated yield; for the oxidation of 11 with 5.0 equiv of TFD: 42.0% substrate conversion, 68.1% yield based on the recovered starting material, 28.6% isolated yield; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  7.11 and 6.93 (two d, 1 H, J = 6.5 Hz, Ala-NH), 6.50 (d, 1 H, J = 7.3 Hz, Val-NH), 4.51 (m, 1 H, Ala-C<sup> $\alpha$ </sup>H), 4.30 (m, 1 H, Val-C<sup>\alpha</sup>H), 3.75 and 3.74 (two s, 3 H, OCH<sub>3</sub>), 2.06 and 2.04 (two s, 3 H, C(O)CH<sub>3</sub>), 1.42 (m, 3 H, Ala-CH<sub>3</sub>), 1.33 and 1.18 (two s, 6 H, Val- $C^{\gamma,\gamma'}H_3$ ); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz)  $\delta$  172.8 and 172.5 (C), 171.5 and 171.2 (C), 170.9 and 170.5 (C), 71.8 and 71.4 (Val-C<sup>β</sup>OH), 58.7 and 58.3 (Val-C<sup>α</sup>H), 52.6 (OCH<sub>3</sub>), 48.1 (Ala- $C^{\alpha}$ H), 27.3, 27.2, 25.7 and 25.3 (Val- $C^{\gamma,\gamma'}$ H<sub>3</sub>), 23.1 and 23.0 (C(O)- $CH_3$ ), 17.8 and 17.6 (Ala- $CH_3$ ); HRMS-FAB (M + Na<sup>+</sup>) calculated for C<sub>11</sub>H<sub>20</sub>NaN<sub>2</sub>O<sub>5</sub> 283.1270, found 283.1275.

*N*-Acetyl-γ-hydroxyleucylvaline methyl ester (12a): for the oxidation of 12 with 2.4 equiv of TFD: 48.2% isolated yield; for the oxidation of 12 with 5.0 equiv of TFD: 95.0% substrate conversion, 59.7% yield based on the recovered starting material, 59.7% isolated yield; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 7.36 (d, 1 H, J = 8.6 Hz, Val-NH), 6.88 (d, 1 H, J = 6.7 Hz, Leu-NH), 4.62 (q, 1 H, J = 6.4 Hz, Leu-C<sup>α</sup>H), 4.45 (m, 1 H, Val-C<sup>α</sup>H), 3.72 (s, 3 H, OCH<sub>3</sub>), 2.17 (m, 1 H, Val-C<sup>β</sup>H), 2.05 and 1.81 (two m, 2 H, Leu-C<sup>Δ,δ</sup>/H<sub>3</sub>), 0.92 and 0.88 (two d, 6 H, J = 6.9 Hz, Val-C<sup>γ,γ</sup>/H<sub>3</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 172.6 (C), 172.4 (C), 170.4 (C), 70.3 (Leu-C<sup>γ</sup>OH), 57.4 (Val-C<sup>α</sup>H), 30.4 and 29.6 (Leu-C<sup>Δ,δ</sup>/H<sub>3</sub>), 23.2 (C(O)CH<sub>3</sub>), 19.0 and 17.6 (Val-C<sup>γ,γ</sup>/H<sub>3</sub>); HRMS-FAB (M + Na<sup>+</sup>) calculated for C<sub>14</sub>H<sub>26</sub>NaN<sub>2</sub>O<sub>5</sub> 325.1739, found 325.1728.

**2-**(*N*-acetyl-valinylamino)-4,4-dimethyl-4-butanolide (13a): 51.5% substrate conversion, 69.9% yield based on the recovered starting material, 36.0% isolated yield; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  7.63 (d, 1 H, *J* = 7.3 Hz, C<sup>2</sup>N*H*), 6.60 (d, 1 H, *J* = 8.9 Hz, Val-N*H*), 4.76 (m, 1 H, C<sup>2</sup>*H*), 4.38 (m, 1 H, Val-C<sup>α</sup>*H*), 2.51 and 2.07 (two m, 3 H, C*H*<sub>2</sub> and Val-C<sup>β</sup>*H*), 2.00 (s, 3 H, C(O)C*H*<sub>3</sub>), 1.49 and 1.42 (two s, 6 H, C*H*<sub>3</sub>), 0.98 and 0.95 (two d, 6 H, *J* = 6.8, Val-C<sup>γ,γ'</sup>*H*<sub>3</sub>), <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz)  $\delta$  174.1 (*C*), 172.0 (*C*), 170.4 (*C*), 82.2 (*C*<sup>4</sup>), 58.2 (Val-C<sup>α</sup>H), 50.0 (*C*<sup>2</sup>H), 41.0 (*CH*<sub>2</sub>), 31.3 (Val-C<sup>β</sup>*H*), 28.8 and 27.1 (two CH<sub>3</sub>), 23.1 (C(O)CH<sub>3</sub>), 19.1 and 18.4 (Val-C<sup>γ,γ'</sup>H<sub>3</sub>), HRMS-FAB (M + Na<sup>+</sup>) calculated for C<sub>13</sub>H<sub>22</sub>NaN<sub>2</sub>O<sub>4</sub> 293.1477, found 293.1479.

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**Supporting Information Available:** Experimental details and supplemental characterization data of starting materials and the oxidation products. This material is available free of charge via the Internet at http://pubs.acs.org.

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