A New and Efficient Synthesis of Unnatural Amino Acids and Peptides by Selective 3,3-Dimethyldioxirane Side-Chain Oxidation

Raffaele Saladino,*,† Maurizio Mezzetti,† Enrico Mincione,† Ines Torrini,‡ Mario Paglialunga Paradisi,‡ and Gaia Mastropietro‡

Dipartimento A.B.A.C., Università degli studi della Tuscia, Via S. Camillo de Lellis, 01100 Viterbo, Italy, and Dipartimento di Studi Farmaceutici e Centro di Studio per la Chimica del Farmaco del CNR, Università "La Sapienza", 00185 Roma, Italy

Received February 1, 1999

N-Boc derivatives of Leu, Met, Thr, Trp, and Pro, the properties of which resemble those of the respective α-amino acid residues present in proteins, rapidly oxidize in the presence of 3,3-dimethyldioxirane to give different products depending on the structure of the oxidizable group in the side chain. A high regioselectivity for the oxygen atom insertion into the γ-CH bond of Leu residues with respect to the weaker α-CH bond was observed. A position selectivity in the oxidation of peptides containing more than one Leu residue was also found.

Introduction

The key role of proteins and their constituent α-amino acids in the structure and function of living matter has stimulated extensive study of these compounds and their analogoues. In particular, the possibility to synthesize unnatural amino acids and peptides is of increasing interest for the development of an efficient and sustainable chemical engineering of proteins.[1] Several methods have been developed in recent years to overcome the poor stability and the lack of oral absorption in the use of peptides as therapeutic agents. These methods are classified according to whether side-chain alterations or backbone modifications are obtained. Backbone modifications include changes at any one of the three characteristic repeating NH, CO, and α-CH elements.[2] Side-chain modifications are in general obtained by oxidation of relatively low redox potential residues, as, for example, cysteine,[3] methionine,[4] tryptophan,[5] histidine,[6] serine,[7] and tyrosine[8] residues. Despite 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 CH bond present in the side chain of α-amino acids, with the exception of the glycine-selective chemical modification by nickel peroxide,[9] in which case a concomitant backbone modification was observed, probably because of the low reactivity generally shown by this bond. In a recent paper,[10] we have reported the use of 3,3-dimethyldioxirane (DMD)[11] in the synthesis of unnatural α-amino acids and peptides via selective CH bond hydroxylation of leucine derivatives. A noteworthy feature of this study is the high regioselectivity for the γ-CH bond with respect to the weaker α-CH bond. Dimethyldioxirane CH bond hydroxylation has been studied in recent years. The mechanism of this trasformation is a subject of controversy, the main question being whether the reaction is a concerted mechanism via an "oxenoid" intermediate[12] or a radical-pair mechanism.[13] Recently, Rauk and coworkers employed ab initio calculations[14] to study the oxidation of the CH bonds in homo- and heterosubstituted alkanes by DMD as a theoretical model for our hydroxylation of Leu derivatives. This model, which describes adequately the selectivity observed in the oxidation of Leu derivatives, involves a higly polar asynchronous transition state, which is common for either concerted oxygen insertion into the CH bond and formation of a radical pair (alkyl radical + α-hydroxyalkoxyl radical). The agreement with experimental data of Leu oxidation is further improved by taking into account the influence of the dielectric reaction medium. Thus, side-chain CH bonds of protected amino acids and proteins are expected to be more probable points for the attack of DMD.

† Università degli studi della Tuscia.

‡ Università "La Sapienza".

(1) (a) Offord, R. E. *Protein Eng.* **1987**, *1*, 151. (b) Gaetner, H. F.; Offord, R. E.; Cotton, R.; Timms, D.; Camble, R.; Rose, K. *J. Biol. Chem.* **1994**, *269*, 7224−7230. (c) Mutter, M. *Angew Chem., Int. Ed. Engl.* **1985**, *24*, 639. (d) Mutter, M.; Vuilleumier, S. *Angew Chem., Int. Ed. Engl.* **1989**, *28*, 535.

(2) (a) Ranganathan, D.; Vaish, N. K.; Shah, K. *J. Am. Chem. Soc.* **1994**, *116*, 6545−6557. (b) Bol, K. M.; Liskamp, R. M. J. *Tetrahedron Lett.* **1991**, *32*, 5401−5404.

(3) Jungheim, L. N.; Shepherd, T. A.; Baxter, A. J.; Burgess, J.; Hatch, S. D.; Lubbehusen, P.; Wiskerchen, M.; Muesing, M. A. *J. Med. Chem.* **1996**, *39*, 96−108.

(4) (a) Blondelle, S. E.; Perez-Paya, E.; Allicotti, G.; Foored, B.; Houghten, R. A. *Biophys. J.* **1995**, *69*, 604−611. (b) Chowdhury, S.; Eshraghi, J.; Wolfe, H.; Forde, D.; Hlavac, A. G.; Johnston D. *Anal. Chem.* **1995**, *67*, 390−398.

(5) Ranganathan, S.; Ranganathan, D.; Bhattacharyya, D. *J. Chem. Soc., Chem. Commun.* **1987**, 1085−1086.

(6) (a) Ranganathan, S.; Ranganathan, D.; Bhattacharyya, D. *Tetrahedron Lett.* **1991**, *32*, 5615−5618. (b) Veda, J.; Ozawa, T.; Miyazaki, M.; Fujiwara, Y. J. *Inorg. Biochem.* **1994**, *55*, 123−130.

(7) Grammer, J. C.; Loo, J. A.; Edmonds, C. G.; Cremo, C. R.; Yount, R. G. *Biochemistry* **1996**, *35*, 15582−15592.

(8) Barlunga, J.; Garcia-Martin, M. A.; Gonzales, J. M.; Clapès, P.; Valencia, G. *Chem. Commun.* **1996**, 1505.

(9) Easton, C. J.; Eichinger, S. K.; Pitt, M. J. *J. Chem. Soc., Chem. Commun.* **1992**, 1295.

(10) Mezzetti, M.; Mincione, E.; Saladino, R. *Chem. Commun.* **1997**, 1063−1064.

(11) Curci, R. In *Advances in Oxigenated Processes*; Baumstark, A. L., Ed.; JAI: Greenwhich, CT, 1990; Vol. 2, Chapter 1, pp 1−59.

(12) (a) Curci, R.; Dinoi, A.; Fusco, C.; Lillo, M. A. *Tetrahedron Lett.* **1996**, *37*, 249. (b) Asensio, G.; Mello, R.; Gonzales-Nunez, M. E.; Boix, C.; Royo, J. *Tetrahedron Lett.* **1997**, *38*, 2373. (c) Simakov, P. A.; Choi, S. Y.; Newcomb, M. *Tetrahedron Lett.* **1998**, *39*, 8187−8190. (d) Glukhovtsev, M. N.; Canepa, C.; Bach, R. D. *J. Am. Chem. Soc.* **1998**, *120*, 10528. (e) Du, X.; Houk, K. N. *J. Org. Chem.* **1998**, *63*, 6480.

(13) (a) Vanni, R.; Garden, S. J.; Banks, J. T.; Ingold, K. U. *Tetrahedron Lett.* **1995**, *36*, 7999−8002. (b) Bravo, A.; Fontana, F.; Fronza, G.; Minisci, F.; Zhao, L. *J. Org. Chem.* **1998**, *63*, 254 and references therein.

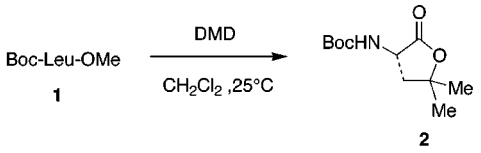
(14) Shustov, G. V.; Rauk, A. *J. Org. Chem.* **1998**, *63*, 5413−5422.

10.1021/jo990185w CCC: $18.00
Published on Web 10/21/1999

In this paper, we wish to report extensively our data on the synthesis of unnatural amino acids and peptides by selective DMD side-chain oxidation of several α-amino acid and peptide derivatives. A high regioselectivity was found in the oxidation of dipeptides and tripeptides containing more than one Leu residue.

Results

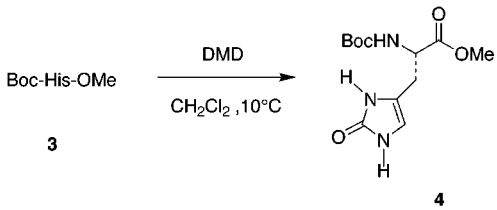
Initially, we tried to study the oxidation of protected Gly, Ala, Val, Ile, Leu, and Phe derivatives as representative examples of α-amino acids bearing an aromatic and aliphatic alkyl side chain. The oxidations were performed with a freshly prepared solution of DMD (0.1 N acetone solution, 6.0 equiv)[15] in CH_2Cl_2 at 25 °C for 3 days. Under these experimental conditions, only the Boc-Leu-OMe (**1**) was found to be reactive. In this case, the 4,4-dimethyl-4-butanolide derivative **2** was obtained as the only recovered product in 93.4% isolated yield (eq 1), with some unreacted substrate (45% substrate conversion).[10] Compound **2** is probably formed by selective oxygen atom insertion into the tertiary γ-CH bond of Leu followed by cyclization.



(1)

A noteworthy feature of this transformation is the high regioselectivity for the γ-CH bond with respect to the weaker α-CH bond and the unexpected stability of the otherwise reactive tertiary CH bond present in the valine substrate.[16] In the latter case, a rationalization based on simple electronic deactivation by the amino functionality on the more proximate tertiary β-CH bond of valine with respect to leucine seems reasonable. This hypothesis is in accord with the recently reported procedure[17] for the selective oxyfuntionalization of unactivated σ-CH bond of alkylamines in acid medium by methyl(trifluoromethyl)dioxirane.

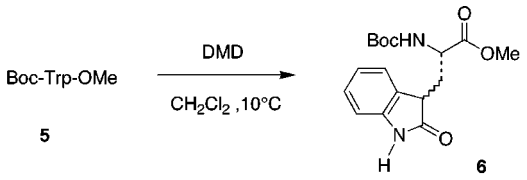
The reactivity of some other representative protected α-amino acids with DMD was then studied. The oxidation of Boc-His-OMe (**3**) afforded Boc-β-(2,3-dihydro-2-oxo-imidazol-4-yl)-alanine methyl ester (**4**) as the only recovered product (59% substrate conversion) in 93.2% isolated yield (eq 2).



(2)

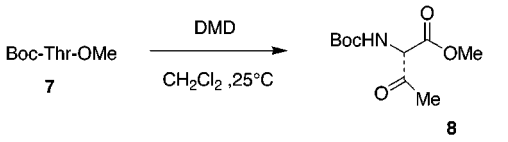
Probably, the oxidation of **3** proceeds, in accord with the results reported by Adam[18] for the oxidation of indoles with DMD, through a reactive oxiranyl intermediate that may in turn rearrange with hydrogen shift to compound **4**. This transformation is a synthetic alternative to the reported ascorbate-mediated oxidation of the imidazole ring in *N*-benzoyl histidine derivatives in which case the corresponding *N*-benzoyl-β-(2-oxo-imidazol-4-yl)-alanine (not shown) was obtained as the main product.[19]

The oxidation of Boc-Trp-OMe (**5**) afforded the Boc-β-(2,3-dihydro-2-oxo-indol-3-yl)alanine methyl ester (**6**) as a chromatographically separable mixture of diastereoisomers (less polar **6a** and more polar **6b** isomers) in 91% total yield (92% substrate conversion; ratio **6a**/**6b** = 1:1) (eq 3). A similar result has been previously obtained in the oxidation of tryptophan residues with the myeloperoxidase, a bacteria-killing enzymatic system, that displays its biological function into phagocytic vacuoles.[20]



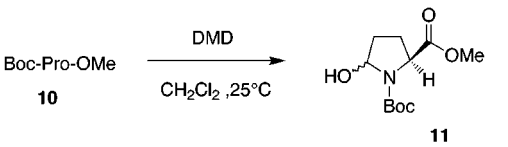
(3)

The transformations reported in eqs 2 and 3 were found to be very selective, and the possible oxidation of the unprotected heterocyclic N−H moiety was not an operative process in our experimental conditions. These data are in accord with results previously reported by us on the DMD oxidation of benzoimidazole, benzothiazole, purine, and pyrimidine derivatives.[21] The oxidation of Boc-Thr-OMe (**7**) performed under similar experimental conditions gave Boc-(*S*)-α-acetylglycine methyl ester (**8**) in 92.4% yield (66% substrate conversion; eq 4). It is noteworthy that the oxidation of Boc-Ser-OMe (**9**) (not shown) did not give products in appreciable amounts, even in the presence of a large excess of DMD and after a longer reaction time.



(4)

The oxidation of Boc-Pro-OMe (**10**) afforded Boc-5-hydroxyproline methyl ester (**11**) as the only recovered product (70% substrate conversion) in 88.6% isolated yield (eq 5).

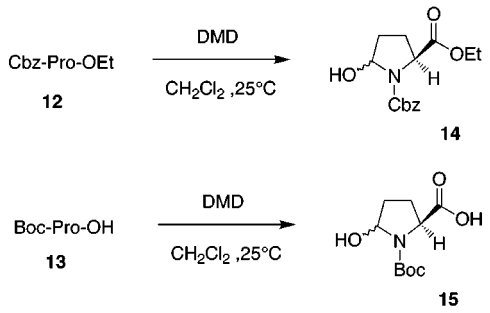


(5)

The structure of compound **11** was unambiguosly assigned on the basis of spectroscopic data. In particular,

(15) Adam, W.; Bialas, J.; Hadjiarapoglou, L. *Chem. Ber.* **1991**, *124*, 2377.
(16) Adam, W.; Curci, R.; Edwards. J. O. *Acc. Chem. Res.* **1989**, *22*, 205.
(17) Asensio, G.; Mello, R.; Gonzales-Nunez, M. E.; Bernardini, C. B.; Adam, W. *J. Am. Chem. Soc.* **1993**, *115*, 7250.
(18) Adam, W.; Ahrweiler, M.; Sauter, M.; Schmiedeskamp, B. *Tetrahedron Lett.* **1993**, *34*, 5247−5250.
(19) Uchida, K.; Kawakishi, S. *Bioorg. Chem.* **1989**, *17*, 330−343.
(20) Drozdz, R.; Naskalski, J. W.; Sznajd, J. *Biochim Biophys. Acta* **1988** *957*, 47−52.
(21) (a) Frachey, G.; Crestini, C.; Bernini, R.; Saladino, R.; Mincione, E. *Heterocycles* **1994**, *38*, 2621−2630. (b) Saladino, R.; Crestini, C.; Bernini, R.; Frachey, G.; Mincione, E. *J. Chem. Soc., Perkin Trans. 1* **1994**, 3053−3054. (c) Saladino, R.; Crestini, C.; Bernini, R.; Mincione, E.; Ciafrino, R. *Tetrahedron Lett.* **1995**, *36*, 2665−2668. (d) Saladino, R.; Crestini, C.; Bernini, R.; Mezzetti, M. *Tetrahedron* **1996**, *52*, 6759−6780.

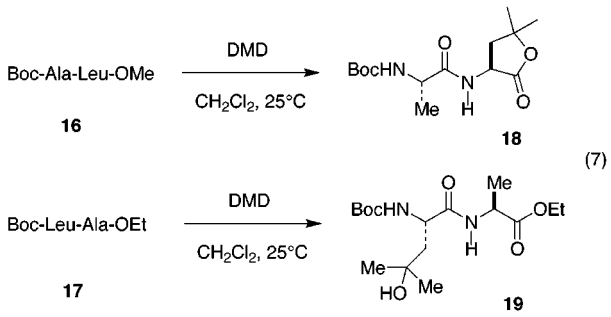
the presence of a methine signal at 5.50 ppm in the ^{1}H NMR spectrum, corresponding to deshielded H-5 proton, and the contemporary presence of a tertiary carbon signal at 82.20 ppm in the ^{13}C NMR spectrum, are diagnostic. A selective oxygen atom insertion at the C-5 position of the pyrrolidine ring was successively observed in the oxidation of Cbz-Pro-OEt (**12**) and Boc-Pro-OH (**13**). The oxidations performed in similar experimental conditions afforded Cbz- or Boc-5-hydroxyproline derivatives **14** and **15** in 89.4% and 92.5% yields, respectively (65% and 53% substrate conversions, respectively; eq 6).



(6)

No aldehydic signals were found in the ^{1}H NMR spectra of compounds **11**, **14**, and **15**, showing that these products are stable enough, in our experimental conditions, to exist only in the hemiaminal form. It is well-known from the literature that the oxidative cleavage of proline derivatives and proline-containing peptides induced by free hydroxyl radicals proceeds via regioselective C-2 hydrogen abstraction to the corresponding 2-pyrrolidone derivatives.[22] In the latter case, the 2-pyrrolidone-5-carboxylic acid (pyroglutamic acid), a product clearly formed by C-5 oxyfunctionalization, was found as a byproduct in very low yield. The highly selective formation of products of C-5 oxygen atom insertion in the oxidations of proline derivatives with DMD (δ-regioselectivity) can be considered as an argument in favor of the concerted mechanism. ^{1}H NMR ($CDCl_3$, 200 MHz) spectra of compounds **2** and **8** were performed in the presence of tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorato]europium(III) [Eu(hfc)$_3$] or tris[3-(2,2,2-trifluoro-1-hydroxyethylidene)-*d*-camphorato]europium-(III) [Eu(tfc)$_3$] as selected examples to evaluate the optical purity of the monoamino acid derivatives. After the addition of the chiral shift reagents (from 50 to 200 μL of a 1 M $CDCl_3$ solution), we detected an enantiomeric excess higher than 98.0%.

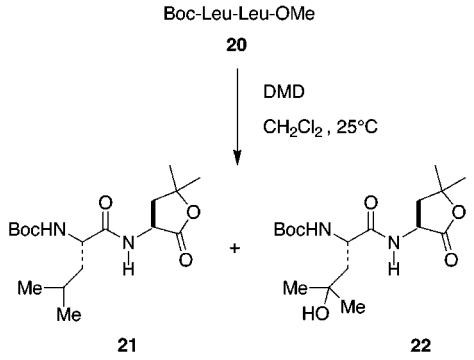
The feasibility of trasformation of leucine derivatives by DMD with respect to the other α-amino acids bearing an aliphatic or aromatic alkyl side-chain promped us to evaluate the possibility to obtain a chemo- and regioselective modification of Leu-containing dipeptides and tripeptides.[23] The oxidation of Boc-Ala-Leu-OMe (**16**) and Boc-Leu-Ala-OEt (**17**) afforded the butanolide derivative **18** and Boc-Leu(γ-OH)-Ala-OEt (**19**) in 87.5% and 81% isolated yields, respectively, with unreacted substrates (40% and 47% substrate conversions, respectively; eq 7).



(7)

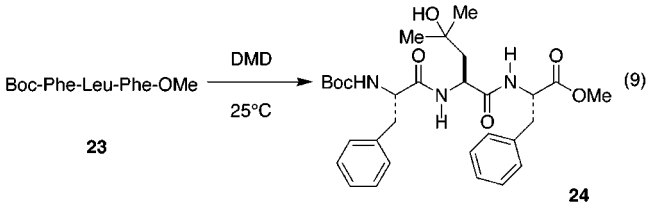
Compound **18**, which is obtained by modification of the Leu residue, may be formed by selective oxygen atom insertion into the tertiary γ-bond, followed by spontaneous cyclization. On the other hand, the presence of a free alcoholic moiety in **19** (as shown by the OH group absorption at 3390 cm^{-1} 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.

The oxidation of the dipeptide Boc-Leu-Leu-OMe **20**, bearing two reactive Leu residues, with DMD (6.0 equiv) in CH_2Cl_2 at 25 °C for 3 days afforded the butanolide derivative **21** in 78.6% isolated yield (42% substrate conversion; eq 8). Traces of **22** (<4%) were also formed, as shown by ^{1}H NMR spectral analyses of the crude reaction mixture.



(8)

The oxidation of the peptide Boc-Phe-Leu-Phe-OMe (**23**) was then studied as an example of oxidation of a tripeptide containing one Leu residue. The oxidation of **23** gave Boc-Phe-Leu(γ-OH)-Phe-OMe (**24**) in 96.3% yield as the only recovered product (51% substrate conversion; eq 9).

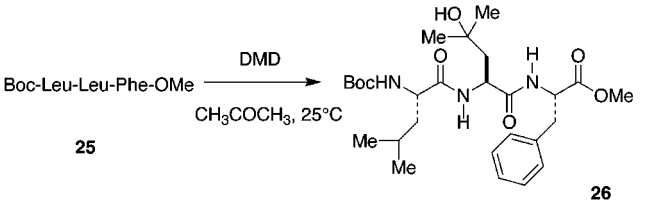


(9)

Tripeptides bearing two Leu and one Phe residues, namely Boc-Leu-Leu-Phe-OMe (**25**), Boc-Leu-Phe-Leu-OMe (**27**), and Boc-Phe-Leu-Leu-OMe (**29**), were successively oxidized to study the reactivity of the Leu residue toward DMD in relation to its position in the peptide. The oxidation of **25** afforded Boc-Leu-Leu(γ-OH)-Phe-OMe (**26**) in 95.5% isolated yield (eq 10) in addition to unreacted substrate (45% substrate conversion).

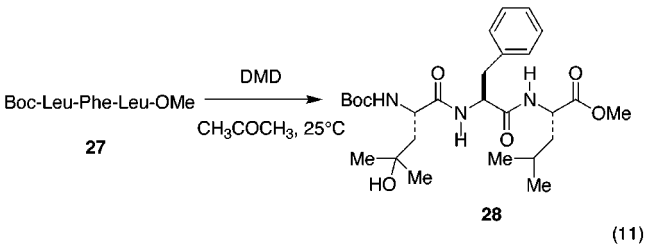
(22) (a) Miller, E. J.; Gay, S. *Methods Enzimol.* **1982**, *82*, 3−32. (b) Uchida, K.; Kaito, Y.; Kawakishi, S. *Biochem. Biophys. Res. Commun.* **1990**, *169*, 265−271.

(23) Recently, Rosner and co-workers reported the importance of the dileucine motifs in biological systems. In particular, dileucine motifs have been implicated in the internalization of degradation of many membrane proteins: Morrison, P.; Chunhg K. C.; Rosner, M. R. *Biochemistry* **1996**, *35*, 14618.



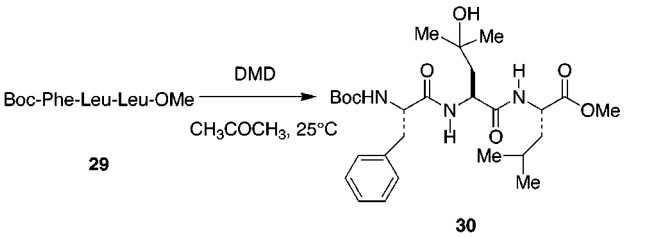
(10)

The oxidation of the tripeptide **27** gave Boc-Leu(γ-OH)-Phe-Leu-OMe (**28**) in 95% isolated yield (43% substrate conversion; eq 11).



(11)

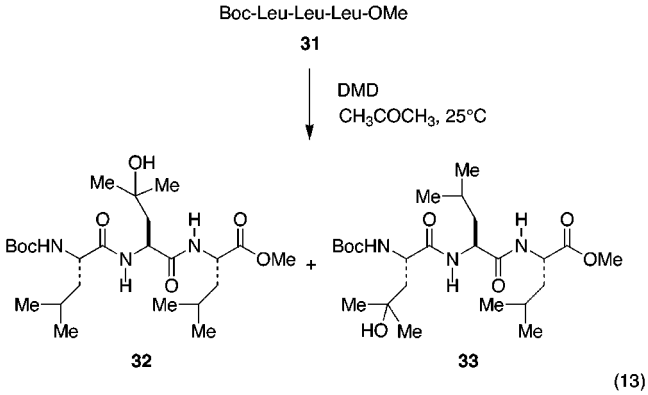
Treatment of **29** with DMD under similar experimental conditions afforded the Boc-Phe-Leu(γ-OH)-Leu-OMe (**30**) in 92.7% isolated yield (41% substrate conversion; eq 12). The structures of compounds **26**, **28**, and **30** were assigned on the basis of ^{1}H NMR experiments. All the signals for the Phe residue were found unchanged, showing that the oxidation of the Leu residue was selective. This result is in accord with the lack of reactivity shown by protected Phe derivatives. In the oxidized peptides **26**, **28**, and **30**, only the β-CH_2 protons of one of two Leu residues resonate, as an AB part of an ABX system, downfield with respect to the signals (multiplets) of both the corresponding groups in the substrate, probably because of the electron-withdrawing effect exerted by the γ-OH group.



(12)

This finding indicates an high position selectivity in the oxidation of peptides containing more than one Leu residue. ^{1}H NMR spin−spin decoupling experiments were successively performed to evaluate the position (N-terminal versus central or C-terminal position) of the modified Leu residue in tripeptides **26** and **28**. This assignment was made on the basis of the knowledge that, inside a group of structurally related peptides, the urethane NH and the adjacent α-CH resonate upfield as compared with the amide NH and the associated α-CH protons, respectively. Thus, the irradiation of the α-CH group of the modified Leu residue causes the collapse of the amide (in **26**) or urethane (in **28**) NH doublet to a singlet and the modification of the β-CH_2 AB part of the ABX to an AB quartet. The position of the oxidized residue in **30**, which does not contain N-terminal Leu, was established on the basis of the detection of the COOMe resonance and of the lack of the characteristic signal pattern expected for the formation of the butanolide derivative, generally obtained after γ-CH bond oxygen atom insertion into the *C*-terminal Leu residue.

Finally, the oxidation of the tripeptide Boc-Leu-Leu-Leu-OMe (**31**) bearing three reactive Leu residues was studied. In this case, the Leu side-chain modified peptides **32** and **33** were obtained in 65.6% and 21% yields (61% substrate conversion), respectively (eq 13). The structure assignment to derivatives **32** and **33** was made as previously described for oxidized peptides containing two Leu residues.



(13)

Conclusion

This study shows that *N*-Boc derivatives of Leu, Thr, Trps and Pro, the properties of which resemble the properties of the respective α-amino acid residues present in protein structures, rapidly oxidize in the presence of DMD acetone solution. The reaction products depend on the structure of the oxidizable group in the side chain of the amino acid residue. In particular, the feasibility of transformation of Boc-Leu-OMe (**1**) by DMD with respect to the other α-amino acids bearing an aliphatic and aromatic alkyl side chain (Boc-Gly-OMe, Boc-Ala-OMe, Boc-Val-OMe, Boc-Ile-OMe, Boc-Phe-OMe) offers a novel synthetic route to side-chain modified peptides or proteins without backbone modifications. A noteworthy feature of this transformation is the high regioselectivity for the γ-CH bond with respect to the weaker α-CH bond and β-CH in Phe residue. In this context, the reactivity of the Leu toward DMD appears to be extremely sensitive to the position of the residue into the peptide. In particular, C-terminal Leu residues are more reactive than N-terminal residues toward DMD in the oxidation of dipeptide **20**. This reaction pattern is completely switched in the oxidation of tripeptides containing two or three Leu residues where the central Leu is selectively oxidized. In the case of the tripeptide Boc-Leu-Leu-Leu-OMe (**31**), the oxidation of the N-terminal Leu was also found to be operative in low amounts. Moreover, the oxidation of N-terminal Leu becomes the main transformation when tripeptide **27**, bearing a low reactive Phe residue at the central position, was oxidized. It is noteworthy that the oxidation of the C-terminal Leu residue of tripeptides was not operative even in the presence of a large excess of DMD and for a longer reaction time. Neither the aromatic or the benzylic positions of the Phe residue present in tripeptides **25**, **27**, **29**, and **31** were found to be reactive under our experimental conditions. However, even if the efficiency of DMD in the insertion of oxygen into tertiary σ-CH

bonds has been widely reviewed in recent works,[24] to the best of our knowledge it is reported in the literature that only activated aromatic rings[25] and doubly activated secondary benzylic positions (the activating effect being exerted by alcoholic or ethereal oxygens, or by a second benzene moiety) are reactive toward DMD.[26] The "apparent" protection of Leu residues after the oxyfunctionalization of a first central or N-terminal Leu cannot be easy explained on the basis of data so far available about the mechanism of DMD σ-CH bond oxygen atom insertion. DMD has been well established to be extremely sensitive to solvent, polar, and stereoelectronic effects.[27] In particular, a polar group close to the reactive center may influence the reaction, favoring a chemo- and stereospecific attack[28] or preventing the oxidation.[29] In the latter case, an example of "apparent" protection of potentially reactive OH moieties in the oxidation of diol derivatives, probably due to a new formed carbonyl moiety, has been reported.[30] Moreover, the high regioselectivity observed in the oxidation of cyclic polyols compared with that for open chain ones has been explained on the basis of conformational considerations.[30] These data suggest that, after the oxidation of the first Leu residue, deactivating effects against the geometrical DMD approach to substrate might be due to new conformational peptide states (in this case the newly introduced OH group might play an important role). The high sequence selectivity shown by DMD in the side-chain oxidation of dipeptide and tripeptide models can be considered, at least in some general aspects, similar to the high sequence selectivity usually shown in the biological trasformations of important peptides and proteins into the cell. Thus, one may suggest that DMD might readily interact with a number of proteins in a sequence-specific manner producing side-chain modified derivatives.

Work is in progress in our laboratories to obtain more experimental evidence about the side-chain oxidation selectivity of biologically important peptides by DMD, as well as the conformational analysis of modified peptides.

Experimental Section

General Methods. Descriptions of analytical instruments and ^{1}H NMR and IR spectrometers have been previously published.[10] Mass spectra were recorded on a spectrometer with an electron beam of 70 eV. Chromatographic purifications were performed on columns packed with silica gel, 230−400 mesh, for flash tecnique. Thin-layer chromatographies were carried out using platten Kieselgel 60 F254. $[\alpha]_D$ measurements were performed by a digital polarimeter. All reagents and solvents were of the highest grade commercially available and used purified or freshly distilled as required by literature procedures.

Synthesis of Starting Materials. General Procedures. Boc-amino Esters. Method A. A 1 mmol portion of L-amino acid methyl ester hydrochloride (Leu, His, Gly, Ala, Ile, Val, Ser, Phe) was dissolved in a 50% (v/v) solution of triethylamine (6 mL) in methanol at 25 °C. To this mixture was added 2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile (Boc-ON) (2 equiv) with vigorous stirring. The reaction was kept at 25 °C for 24 h. The solvent was evaporated under reduced pressure and purified by flash chromatography on silica gel eluting with a gradient petroleum ether/ethyl acetate as elution solvent.

Method B. To a stirred solution of 1 mmol of Boc-L-amino acid (Trp, Met, Thr, Pro) in 5 mL of MeOH was added a large excess of a ethereal solution of CH_2N_2 at 0 °C. The reaction mixture was evaporated under reduced pressure. The crude was purified by flash chromatography on silica gel using 9:1 CH_2Cl_2/CH_3OH as the elution solvent.

Boc-dipeptide Esters. To a stirred solution of 1 mmol of the unprotected dipeptide in 10 mL of dry MeOH at −5 °C was added carefully $SOCl_2$ (2 equiv). The reaction mixture was stirred at 20 °C for 2 h and at 80 °C for 1.5 h. The solvent was evaporated under reduced pressure. The crude was purified by flash chromatography on silica gel using 8:2 CH_2Cl_2/CH_3OH as elution solvent. Dipeptide methyl esters were dissolved in a 50% (v/v) solution of triethylamine (6 mL) in methanol at 25 °C. To this stirred mixture was added Boc-ON (2 equiv), and stirring was continued at 25 °C for 24 h. The reaction mixture was evaporated under reduced pressure and purified by flash chromatography on silica gel using a gradient petroleum ether/ethyl acetate as elution solvent. The Boc-Leu-Ala-OEt (**17**) was prepared starting from commercially available Boc-Leu-Ala-OH and dissolved in 10 mL of Et_2O and 0.5 mL of EtOH in the presence of *N*,*N*-dicyclohexylcarbodiimide (2 equiv) and a catalytic amount of 4-(dimethylamino)pyridine, and the mixture was stirred at 25 °C for 24 h. The solvent was evaporated under reduced pressure, and the crude was purified by flash chromatography on silica gel using a gradient CH_2Cl_2/CH_3OH as the elution solvent. Spectroscopic data of Boc-amino esters and Boc-dipeptide esters are reported as material available.

Tripeptides. Boc-Leu-Leu-Phe-OMe (**25**) and Boc-Leu-Phe-Leu-OMe (**27**) were prepared using the mixed anhydride method with isobutyl chloroformate starting from Boc-Leu-OH and TFA·H-Leu-Phe-OMe and TFA·H-Phe-Leu-OMe, respectively.

The trifluoroacetates were obtained by acidolysis of dipeptides Boc-Leu-Phe-OMe[31] and Boc-Phe-Leu-OMe[32] with trifluoroacetic acid.[33]

Boc-Leu-Leu-Phe-OMe (25): mp 127−129 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) 0.80−1.02 (m, 12H), 1.44 (s, 9H), 1.47−1.73 (m, 6H), 3.10 (m, 2H), 3.70 (s, 3H), 4.08 (m, 1H), 4.42 (m, 1H), 4.82 (m, 1H), 4.95 (d, 1H, J = 7.2 Hz), 6.57 (d, 1H, J = 8.1 Hz), 6.64 (d, 1H, J = 7.7 Hz), 7.03−7.33 (m, 5H). ^{13}C NMR ($CDCl_3$, 50 MHz): 21.87 (CH_3), 22.88 (CH_3), 24.57 (CH_3), 24.71 (CH_3), 28.26 (CH_3), 37.84 (CH_2), 40.65 (CH_2), 40.95 (CH_2), 51.65 (CH), 52.25 (CH_3), 53.12 (CH), 53.25 (CH), 80.29 (C), 127.13 (CH), 128.57 (CH), 129.23 (CH), 135.71 (C), 155.72 (C), 171.33 (C), 171.58 (C), 172.50 (C). $[\alpha]_D$ = −23.0° (*c* 1.0, $CHCl_3$); MS-(EI) 505 (M^+, 18). Anal. Calcd for $C_{27}H_{43}N_3O_6$: C, 64.13; H, 8.57; N, 8.31. Found: C, 64.59; H, 8.51; N, 8.24.

Boc-Leu-Phe-Leu-OMe (27): mp 142−144 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) 0.80−1.02 (m, 12H), 1.41 (s, 9H), 1.45−1.72 (m, 6H), 3.08 (m, 2H), 3.69 (s, 3H), 4.07 (m, 1H), 4.52 (m, 1H), 4.73 (m, 1H), 4.94 (br s, 1H), 6.62 (br s, 1H), 6.79 (d, 1H, J = 7.7 Hz), 7.10−7.35 (m, 5H). ^{13}C NMR ($CDCl_3$, 50 MHz) 21.76

(24) (a) Murray, R. W.; Gu, H. *J. Org. Chem*. **1995**, *60*, 5673−5677. (b) Curci, R.; Dinoi, A.; Rubino, M. F. *Pure Appl. Chem*. **1995**, *67*, 818−822 and refs therein.

(25) (a) Murray, R. W.; Singh, M.; Rath, N. P. *J. Org. Chem*. **1996**, *61*, 7660−7661. (b) Adam, W.; Schonberger, A. *Tetrahedron Lett*. **1992**, **33**, 53−56. (c) Crandall, J. K.; Zucco, M.; Kirsch, S.; Coppert, P. M. *Tetrahedron Lett*. **1991**, *32*, 5441−5444. (d) Altamura, A.; Fusco, C.; D'Accolti, L.; Mello, R.; Prencipe, T.; Curci, R. *Tetrahedron Lett*. **1991**, *32*, 5444−5448.

(26) Bovicelli, P.; Sanetti, A.; Bernini, R.; Lupattelli, P. *Tetrahedron* **1997**, *53*, 9755−9760.

(27) Murray, R. W.; Gu, D. *J. Chem. Soc., Perkin Trans 2*. **1994**, 451.

(28) Ballini, R.; Papa, F.; Bovicelli, P. *Tetrahedron Lett*. **1996**, *37*, 3507.

(29) Bovicelli, P.; Lupattelli, P.; Mincione, E.; Fiorini, V. *Tetrahedron Lett*. **1993**, *34*, 6103.

(30) Bovicelli, P.; Truppa, D.; Sanetti, A.; Bernini, R.; Lupattelli, P. *Tetrahedron* **1998**, *54*, 14301.

(31) Sauvè, G.; Rao, V. S.; Lajoie, G.; Belleau, B. *Can. J. Chem*. **1985**, *63*, 3089−3101.

(32) Kessler, H.; Holzemann, G. *Liebigs Ann. Chem*. **1981**, 2028−2044.

(33) Torrini, I.; Pagani Zecchini, G.; Paglialunga Paradisi, M.; Lucente, G.; Gavuzzo, E.; Mazza, F.; Pochetti, G.; Spisani, S.; Giuliani, A. L. *Int. J. Peptide Protein Res*. **1991**, *38*, 495−504.

(CH_3), 21.81 (CH_3), 22.61 (CH_3), 22.84 (CH_3), 24,67 (CH), 28.21 (CH_3), 37.91 (CH_2), 41.21 (CH_2), 50.81 (CH), 52.06 (CH_3), 53.57 (CH), 53.98 (CH), 80.09 (C), 126.82 (CH), 128.45 (CH), 129.32 (CH), 136.39 (C), 155.50 (C), 170.40 (C), 172.42 (C), 172.59 (C); $[\alpha]_D = -48.0°$ (*c* 1.0, $CHCl_3$); MS(EI) 505 (M^+, 22). Anal. Calcd for $C_{27}H_{43}N_3O_6$: C, 64.13; H, 8.57; N, 8.31. Found: C, 64.35; H, 8.51; N, 8.27.

Boc-Phe-Leu-Leu-OMe (**29**) was prepared by coupling of Boc-Phe-Leu-OH, obtained by methanolic alkaline hydrolysis of dipeptide Boc-Phe-Leu-OM,[32] and HCl·H-Leu-OMe by the mixed anhydride method with isobutyl chloroformate.

Boc-Phe-Leu-Leu-OMe (29): mp 155−156 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) 0.83−1.04 (m, 12H), 1.39 (s, 9H), 1.40−1.76 (m, 6H), 3.06 (m, 2H), 3.73 (s, 3H), 4.32−4.64 (m, 3H), 5.09 (d, 1H, $J = 7.8$ Hz), 6.60 (d, 1H, $J = 8.2$ Hz), 6.68 (d, 1H, $J = 8.1$ Hz), 7.13−7.37 (m, 5H); ^{13}C NMR ($CDCl_3$, 50 MHz) 21.84 (CH_3), 22.05 (CH_3), 22.69 (CH_3), 22.74 (CH_3), 24.44 (CH), 28.13 (CH_3), 37.89 (CH_2), 40.96 (CH_2), 41.14 (CH_2), 50.70 (CH), 51.64 (CH_3), 52.15 (CH), 55.64 (CH), 80.25 (C), 126.87 (CH), 128.56 (CH), 129.24 (CH), 136.44 (C), 155.40 (C), 171.38 (C), 173.00 (C); $[\alpha]_D = -64°$ (*c* 2.5, $CHCl_3$). MS(EI) 505 (M^+, 20). Anal. Calcd for $C_{27}H_{43}N_3O_6$: C, 64.13; H, 8.57; N, 8.31. Found: C, 64.43; H, 8.51; N, 8.52.

Boc-Phe-Leu-Phe-OMe (**23**) and Boc-Leu-Leu-Leu-OMe (**31**) were synthesized as reported in refs 34 and 35, respectively.

Oxidation of Boc-amino Esters and Boc-peptide Esters. General Procedure. The oxidations were carried out by adding a freshly prepared solution of 6 equiv of 3,3-dimethyldioxirane (0,1 N acetone solution) (2.5 equiv for Boc-His-OMe **3** and Boc-Trp-OMe **5**; 4 equiv for Boc-Pro-OMe **10**, Cbz- Pro-OEt **12**, Boc-Pro-OH **13**, Boc-Ala-Leu-OMe **16**, and Boc-Leu-Ala-OEt **17**), to the appropriate substrate (1 mmol) in 5 mL of CH_2Cl_2 (Boc-amino acid and Boc-dipeptide esters) or acetone (Boc-tripeptide esters) at 25 °C (10 °C for Boc-His-OMe 3 and Boc-Trp-OMe 5) for 3 days (2 days for **3** and **5**). The DMD excess and the reaction solvent were evaporated under reduced pressure and the crude purified by flash chromatography on silica gel usinga gradient petroleum ether/ethyl acetate as the elution solvent.

(2*S*)-2-(*tert*-Butoxycarbonylamino)-4,4-dimethyl-4-butanolide (2): 45% substrate conversion; 96.2 mg; 93.4%); oil; ^{1}H NMR ($CDCl_3$, 200 MHz) 1.41 (s, 3H), 1.43 (s, 9H), 1.47 (s, 3H), 1.88−2.80 (m, 2H), 4.42−4.62 (m, 1H), 4.95−5.09 (br s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) 26.26 (CH_3), 28.87 (CH_3), 28.98 (CH_3), 42.68 (CH_2), 51.35 (CH), 80.02 (C), 82.51 (C), 156.03 (C), 173.25 (C); $[\alpha]_D = +13.0°$ (*c* 1.0, $CHCl_3$); MS(EI) 229 (M^+, 15). Anal. Calcd for $C_{11}H_{19}NO_4$: C, 57.63; H, 8.35; N, 6.11. Found: C, 57.83; H, 8.40; N, 6.32.

Boc-*β*-(2,3-dihydro-2-oxo-imidazol-4-yl)-alanine methyl ester (4): 59% substrate conversion; 156.7 mg; 93.2%; oil; ^{1}H NMR ($CDCl_3$, 200 MHz) 1.39 (s, 9H), 2.95−3.05 (m, 2H), 3.71 (s, 3H), 4.55−4.85 (m, 1H), 5.32−5.49 (br s, 1H), 7.20 (m, 1H), 8.98 (br s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) 28.00 (CH_3), 31.05 (CH_2), 50.70 (CH), 52.47 (CH_3), 80.39 (C), 85.46 (C), 158.15 (CH), 162.51 (C), 163.50 (C), 171.27 (C); $[\alpha]_D = +13.6°$ (*c* 1.8, $CHCl_3$). MS(EI) 285 (M^+, 21). Anal. Calcd for $C_{12}H_{19}N_3O_5$: C, 50.52; H, 6.71; N, 14.73. Found: C, 50.34; H, 6.92; N, 14.79.

Boc-*β*-(2,3-dihydro-2-oxo-indol-3-yl)-alanine methyl ester (6). 6a: 92% substrate conversion; 140.0 mg; 45.6%; mp 180−182 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) 1.38 (s, 9H), 2.39−2.57 (m, 2H), 3.76 (s, 3H), 4.19−4.32 (m, 1H), 5.44 (s, 1H), 6.59−6.62 (m, 1H), 6.63−6.84 (m, 1H), 7.09−7.20 (m, 1H), 7.21−7.31 (m, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz) 28.16 (CH_3), 41.36 (CH_2), 52.34 (CH_3), 59.77 (CH), 81.15 (C), 84.33 (CH), 110.52 (CH), 119.44 (CH), 123.15 (CH), 130.27 (CH), 148.32 (C), 154.12 (C), 173.90 (C), 175.21 (C); $[\alpha]_D = -221.4°$ (*c* 1.0, $CHCl_3$). MS(EI) 334 (M^+, 18). Anal. Calcd for $C_{17}H_{22}N_2O_5$: C, 61.06; H, 6.63; N, 8.38. Found: C, 61.0; H, 6.59; N, 8.15.

6b: 92% substrate conversion; 140.0 mg; 45.6%; mp 154−156 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) 1.42 (s, 9H), 2.57−2.64 (m, 2H), 3.64 (s, 3H), 4.58−4.78 (m, 1H), 5.30−5.45 (m, 1H), 6.88−7.05 (m, 2H), 7.12−7.32 (m, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz) 28.16 (CH_3), 42.05 (CH_2), 52.21 (CH_3), 61.76 (CH), 81.64 (C), 98.63 (CH), 115.14 (CH), 121.89 (CH), 123.15 (CH), 130.20 (CH), 149.09 (C), 154.52 (C), 172.37 (C), 173.90 (C); $[\alpha]_D = +83.6°$ (*c* 1.0, $CHCl_3$). MS(EI) 334 (M^+, 16). Anal. Calcd for $C_{17}H_{22}N_2O_5$: C, 61.06; H, 6.63; N, 8.38. Found: C, 61.10; H, 6.63; N, 8.19.

Boc-(s)-α-acetylglycine methyl ester (8): 66% substrate conversion; 140.9 mg; 92.4%; mp 63−65 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) 1.41 (s, 9H), 2.23 (s, 3H), 3.80 (s, 3H), 5.15−5.25 (br s, 1H), 6.35−6.48 (br s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) 23.12 (CH_3), 28.08 (CH_3), 53.98 (CH_3), 81.12 (CH), 84.02 (C), 156.0 (C), 168.13 (C), 198.56 (C); $[\alpha]_D = -4.3°$ (*c* 2.8, $CHCl_3$); MS(EI) 231 (M^+, 11). Anal. Calcd for $C_{10}H_{17}NO_5$: C, 51.95; H, 7.40; N, 6.06. Found: C, 52.11; H, 7.36; N, 6.18.

Boc-5-hydroxyproline methyl ester (11): 70% substrate conversion; 151.9 mg; 88.6%; oil; ^{1}H NMR ($CDCl_3$, 200 MHz) 1.36 (s, 9H), 1.78−2.65 (m, 4H), 3.69 (s, 3H), 4.14−4.41 (m, 1H), 5.36−5.65 (m, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) 26.87 (CH_2), 28.17 (CH_3), 31.01 (CH_2), 52.01 (CH_3), 58.94 (CH), 80.69 (C), 82.19 (CH), 153.37 (C), 172.87 (C); $[\alpha]_D = -205.3°$ (*c* 1.5, $CHCl_3$); MS(EI) 245 (M^+, 15). Anal. Calcd for $C_{11}H_{19}NO_5$: C, 53.88; H, 7.80; N, 5.71. Found: C, 53.75; H, 7.78; N, 5.90.

Cbz-5-hydroxyproline ethyl ester (14): 65% substrate conversion; 170.2 mg; 89.4%; oil; ^{1}H NMR ($CDCl_3$, 200 MHz) 1.12 (t, 3H, $J = 8.0$ Hz), 1.82−2.59 (m, 4H), 3.94−4.52 (m, 3H), 5.13 (m, 2H), 5.51−5.76 (m, 1H), 7.12−7.41 (m, 5H); ^{13}C NMR ($CDCl_3$, 50 MHz) 14.04 (CH_3), 27.95 (CH_2), 30.93 (CH_2), 59.12 (CH), 61.16 (CH_2), 67.21 (CH_2), 82.69 (CH), 127.91 (CH), 128.07 (CH), 128.44 (CH), 128.56 (CH), 128.67 (CH), 136.96 (C), 153.94 (C), 172.03 (C); $[\alpha]_D = -82°$ (*c* 2.0, $CHCl_3$); MS(EI) 293 (M^+, 27). Anal. Calcd for $C_{15}H_{19}NO_5$: C, 61.43; H, 6.53; N, 4.77. Found: C, 61.38; H, 6.50; N, 4.92.

Boc-5-hydroxyproline (15): 53% substrate conversion; 113.2 mg; 92.5%; oil; ^{1}H NMR ($CDCl_3$, 200 MHz) 1.42 (s, 9H), 1.74−2.38 (m, 4H), 4.10−4.24 (m, 1H), 5.05−5.24 (m, 1H), 6.54−6.88 (br s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) 27.93 (CH_3), 29.86 (CH_2), 30.81 (CH_2), 55.85 (CH), 80.75 (C), 89.17 (CH), 154.19 (C), 154.28 (C); $[\alpha]_D = +13.5°$ (*c* 2.5, $CHCl_3$). MS(EI) 231 (M^+, 21). Anal. Calcd for $C_{10}H_{17}NO_5$: C, 51.94; H, 7.40; N, 6.06. Found: C, 52.13; H, 7.41; N, 6.10.

(2*S*)-2-(Boc-alanylamino)-4,4-dimethyl-4-butanolide (18): 40% substrate conversion; 105 mg; 87.5%; mp 170−172 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) 1.23 (s, 3H), 1.33−1.37 (m, 3H), 1.41 (s, 9H), 1.48 (s, 3H), 1.88−2.00 (m, 1H), 2.58−2.68 (m, 1H), 4.09−4.20 (m, 1H), 4.65−4.79 (m, 1H), 4.94−4.97 (br s, 1H), 6.77−6.80 (br s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) 18.06 (CH_3), 27.05 (CH_3), 28.28 (CH_3), 28.92 (CH_3), 41.91 (CH_2), 50.28 (CH), 51.30 (CH), 80.0 (C), 82.50 (C), 156.03 (C), 173.18 (C), 174.08 (C); $[\alpha]_D = +15.7°$ (*c* 1.3, $CHCl_3$). MS(EI) 300 (M^+, 15). Anal. Calcd for $C_{14}H_{24}N_2O_5$: C, 56.0; H, 8.0; N, 9.33. Found: C, 56.22; H, 8.12; N, 9.24.

Boc-Leu(γ-OH)-Ala-OEt (19): 47% substrate conversion; 131.9 mg; 81%; oil; ^{1}H NMR ($CDCl_3$, 200 MHz) 1.15−1.30 (m, 12H), 1.42 (s, 9H), 1.51−1.85 (m, 2H), 4.06−4.30 (m, 2H), 4.35 (m, 1H), 4.48−4.60 (m, 1H), 5.59−5.62 (br s, 1H), 6.99−7.02 (br s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) 14.12 (CH_3), 18.19 (CH_3), 18.26 (CH_3), 28.32 (CH_3), 45.28 (CH_2), 48.20 (CH), 50.10 (CH), 61.53 (CH_2), 70.35 (C), 81.66 (C), 156.20 (C), 172.15 (C), 172.84 (C); $[\alpha]_D = -4.2°$ (*c* 1.1, $CHCl_3$). MS(EI) 346 (M^+, 37). Anal. Calcd for $C_{16}H_{30}N_2O_6$: C, 55.5; H, 8.7; N, 8.0. Found: C, 55.4; H, 8.7; N, 8.22.

(2*S*)-2-(Boc-leucylamino)-4,4-dimethyl-4-butanolide (21): 42% substrate conversion; 112.9 mg; 78.6%; oil; ^{1}H NMR ($CDCl_3$, 200 MHz) 0.91−0.94 (m, 6H), 1.23 (s, 3H), 1.41 (s, 9H), 1.48 (s, 3H), 1.48−1.80 (m, 3H), 1.88−2.02 (m, 1H), 2.62−2.76 (m, 1H), 4.07−4.18 (m, 1H), 4.63−4.77 (m, 1H), 4.88−4.91 (d, 1H, $J = 6.0$ Hz), 6.73−6.76 (br s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) 18.06 (CH_3), 22.97 (CH_3), 24.69 (CH), 27.03 (CH_3), 28.27 (CH_3), 41.06 (CH_2), 41.94 (CH_2), 50.24 (CH), 51.10 (CH), 81.10 (C), 82.55 (C), 155.53 (C), 173.15 (C), 174.05 (C); $[\alpha]_D = +7.8°$ (*c* 1.9, $CHCl_3$); MS(EI) 342 (M^+, 15). Anal. Calcd for

(34) Toniolo, C.; Crisma, M.; Moretto, V.; Freer, R. J.; Becker, E. L. *Biochem. Biophys. Acta* **1990**, *1034*, 67−72.

(35) Shields, J. E.; McDowell, S. T.; Pavlos, J.; Gray, G. R. *J. Am. Chem. Soc.* **1968**, *90*, 3549−3556.

$C_{17}H_{30}N_2O_5$: C, 59.60; H, 8.7; N, 8.18. Found: C, 59.77; H, 8.96; N, 8.32.

(2*S*)-2-[Boc-leucylamino(γ-OH)]-4,4-dimethyl-4-butanolide (22): 42% substrate conversion; 4.5 mg; 3%; oil; ^{1}H NMR ($CDCl_3$, 200 MHz) 0.88−0.95 (m, 6H), 1.25 (s, 3H), 1.44 (s, 9H), 1.50 (s, 3H), 1.41−1.75 (m, 2H), 1.88−2.02 (m, 1H), 2.59−2.75 (m, 1H), 4.11−4.19 (m, 1H), 4.68−4.75 (m, 1H), 4.92−4.98 (m, 1H), 6.75−6.79 (m, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) 18.15 (CH_3), 23.05 (CH_3), 27.15 (CH_3), 28.31 (CH_3), 41.11 (CH_2), 41.98 (CH_2), 50.28 (CH), 51.15 (CH), 81.10 (C), 81.25 (C), 82.55 (C), 155.70 (C), 173.20 (C), 178.15 (C); $[\alpha]_D = +11.8°$ (*c* 2.0, $CHCl_3$); MS(EI) 358 (M^+, 37). Anal. Calcd for $C_{17}H_{30}N_2O_6$: C, 56.97; H, 8.44; N, 7.81. Found: C, 56.89; H, 8.44; N, 7.85.

Boc-Phe-Leu(γ-OH)-Phe-OMe (24): 51% substrate conversion; 272.6 mg; 96.3%; oil; ^{1}H NMR ($CDCl_3$, 200 MHz) 1.18 (s, 3H), 1.22 (s, 3H), 1.38 (s, 9H), 1.70 and 1.88 (part A and B of a ABX system, 2H, J = 5.0, 7.7, 14.5 Hz), 2.92−3.22 (m, 4H), 3.71 (s, 3H), 4.27 (m, 1H), 4.44 (m, 1H), 4.75 (m, 1H), 5.02 (d, 1H, J = 7.3 Hz), 7.00 (br s, 1H), 7.03−7.36 (m, 11H); ^{13}C NMR ($CDCl_3$, 50 MHz) 28.28 (CH_3), 30.27 (CH_3), 37.82 (CH_2), 38.08 (CH_2), 43.9 (CH_2), 51.15 (CH), 52.38 (CH_3), 53.58 (CH), 56.17 (CH), 70.54 (C), 80.52 (C), 127.17 (CH), 128.81 (CH), 129.40 (CH), 135.88 (C), 136.16 (C), 155.87 (C), 171.57 (C), 171.62 (C), 171.9 (C); $[\alpha]_D = -5.0°$ (*c* 1, $CHCl_3$); MS(EI) 555 (M^+, 22). Anal. Calcd for $C_{30}H_{41}N_3O_7$: C, 64.85; H, 7.44; N, 7.56. Found: C, 64.80; H, 7.44; N, 7.60.

Boc-Leu-Leu(γ-OH)-Phe-OMe (26): 45% substrate conversion; 224 mg; 95.5%; oil; ^{1}H NMR ($CDCl_3$, 200 MHz) 0.90 (m, 6H), 1.23 (s, 3H), 1.25 (s, 3H), 1.42 (s, 9H), 1.48−1.72 (m, 3H), 1.83 and 1.96 (m, 2H, part A and B of a ABX system, J = 5.2, 8.4, and 14.5 Hz), 3.10 (m, 2H), 3.71 (s, 3H), 4.0 (m, 1H), 4.50 (m, 1H), 4.80 (m, 1H), 4.91 (d, 1H, J = 7.4 Hz), 6.97−7.37 (m, 7H); ^{13}C NMR ($CDCl_3$, 50 MHz) 21.93 (CH_3), 22.84 (CH_3), 24.70 (CH), 28.28 (CH_3), 29.67 (CH_3), 37.80 (CH_2), 40.9 (CH_2), 43.79 (CH_2), 51.06 (CH), 52.26 (CH_3), 52.92 (CH), 53.40 (CH), 70.43 (C), 80.35 (C), 127.09 (CH), 128.54 (CH), 129.28 (CH), 135.86 (C), 155.98 (C), 171.56 (C), 171.73 (C), 172.96 (C); $[\alpha]_D = -40.0°$ (*c* 0.8, $CHCl_3$); MS(EI) 521 (M^+, 18). Anal. Calcd for $C_{27}H_{43}N_3O_7$: C, 62.18; H, 8.30; N, 8.06. Found: C, 62.29; H, 8.30; N, 8.12.

Boc-Leu(γ-OH)-Phe-Leu-OMe (28): 43% substrate conversion; 213 mg; 95%; oil; ^{1}H NMR ($CDCl_3$, 200 MHz) 0.90 (m, 6H), 1.25 (s, 3H), 1.27 (s, 3H), 1.37 (s, 9H), 1.40−1.65 (m, 3H), 1.68 and 2.00 (part A and B of a ABX system, 2H, J = 6.1, 7.2, 14.5 Hz), 3.05 and 3.24 (part A and B of a ABX system, 2H, J = 6.1, 5.8, 14.0 Hz), 3.70 (s, 3H), 4.15 (m, 1H), 4.54 (m, 1H), 4.75 (m, 1H), 5.84 (d, 1H, J = 5.0 Hz), 6.71 (d, 1H, J = 8.1 Hz), 6.98 (br s, 1H), 7.10−7.35 (m, 5H); ^{13}C NMR ($CDCl_3$, 50 MHz) 21.72 (CH_3), 22.75 (CH_3), 24.55 (CH), 28.27(CH_3), 29.88 (CH_3), 37.23 (CH_2), 40.96 (CH_2), 44.12 (CH_2), 50.89 (CH), 52.17 (CH_3), 52.92 (CH), 53.83 (CH), 70.73 (C), 80.30 (C), 127.05 (CH), 128.68 (CH), 129.41 (CH), 136.36 (C), 155.96 (C), 170.65 (C), 172.49 (C), 173.02 (C); $[\alpha]_D = -35.7°$ (*c* 1.5, $CHCl_3$); MS(EI) 521 (M^+, 23). Anal. Calcd for $C_{27}H_{43}N_3O_7$: C, 62.18; H, 8.30; N, 8.06. Found: C, 62.23; H, 8.25; N, 8.16.

Boc-Phe-Leu(γ-OH)-Leu-OMe (30): 41% substrate conversion; 198 mg; 92.7%; oil; ^{1}H NMR ($CDCl_3$, 200 MHz) 0.90 (m, 6H), 1.22 (s, 3H), 1.26 (s, 3H), 1.36 (s, 9H), 1.43−1.70 (m, 3H), 1.82 and 1.96 (part A and B of a ABX system, 2H, J = 5.7, 7.2, 14.5 Hz), 3.04 (m, 2H), 3.70 (s, 3H), 4.32 (m, 1H), 4.45−4.62 (m, 2H), 5.12 (d, 1H, J = 6.7 Hz), 7.08−7.35 (m, 7H); ^{13}C NMR ($CDCl_3$, 50 MHz) 21.81 (CH_3), 22.82 (CH_3), 24.82 (CH), 28.25 (CH_3), 29.89 (CH_3), 37.52 (CH_2), 40.61 (CH_2), 43.7 (CH_2), 50.66 (CH), 52.05 (CH_3), 55.92 (CH), 63.97 (CH), 70.29 (C), 80.19 (C), 127.04 (CH), 128.58 (CH), 129.20 (CH), 136.31 (C), 155.32 (C), 171.86 (C), 171.88 (C), 173.22 (C); $[\alpha]_D = -19.6°$ (*c* 2.7, $CHCl_3$); MS(EI) 521 (M^+, 20). Anal. Calcd for $C_{27}H_{43}N_3O_7$: C, 62.18; H, 8.30; N, 8.06. Found: C, 62.33; H, 8.31; N, 8.44.

Boc-Leu-Leu(γ-OH)-Leu-OMe (32): 61% substrate conversion; 195.0 mg; 65.6%; oil; ^{1}H NMR ($CDCl_3$, 200 MHz) 0.92 (m, 12H), 1.27 (s, 3H), 1.31 (s, 3H), 1.43 (s, 9H), 1.47−1.77 (m, 6H), 1.90 and 2.02 (part A and B of a ABX system, 2H, J = 5.2, 8, 14.5 Hz), 3.73 (s, 3H), 4.04 (m, 1H), 4.47−4.65 (m, 2H), 4.99 (d, 1H, J = 7.5 Hz), 7.15 (d, 1H, J = 7.5 Hz), 7.30 (d, 1H, J = 8.0 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) 21.64 (CH_3), 22.71 (CH_3), 23.76 (CH), 24.63 (CH), 28.16 (CH_3), 29.37 (CH_3), 30.0 (CH_3), 40.95 (CH_2), 43.10 (CH_2), 43.20 (CH_2), 50.77 (CH), 52.19 (CH_3), 52.96 (CH), 53.05 (CH), 70.29 (C), 80.29 (C), 155.98 (C), 171.73 (C), 172.90 (C), 173.10 (C); $[\alpha]_D = -25.2°$ (*c* 2.0, $CHCl_3$); MS(EI) 487 (M^+, 22). Anal. Calcd for $C_{24}H_{45}N_3O_7$: C, 59.11; H, 9.30; N, 8.62. Found: C, 59.26; H, 9.30; N, 8.73.

Boc-Leu(γ-OH)-Leu-Leu-OMe (33): 61% substrate conversion, 63.0 mg; 21%; oil; ^{1}H NMR ($CDCl_3$, 200 MHz) 0.93 (m, 12H), 1.30 (s, 6H), 1.44 (s, 9H), 1.44−1.70 (m, 6H), 1.72 and 2.09 (part A and B of a ABX system, 2H, J = 8.5, 8.5, 14.5 Hz), 3.71 (s, 3H), 4.25 (m, 1H), 4.40−4.62 (m, 2H), 5.67 (d, 1H, J = 6.8 Hz), 6.71 (br s, 1H), 7.09 (br s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) 21.50 (CH_3), 22.80 (CH_3), 23.06 (CH), 24.63 (CH), 24.69 (CH), 28.25 (CH_3), 29.15 (CH_3), 30.49 (CH_3), 40.43 (CH_2), 40.98 (CH_2), 44.90 (CH_2), 50.62 (CH), 51.68 (CH), 52.16 (CH_3), 70.30 (C), 80.33 (C), 155.65 (C), 172.0 (C), 172.10 (C), 173.0 (C); $[\alpha]_D = -61°$ (*c* 1.0, $CHCl_3$); MS(EI) 487 (M^+, 21). Anal. Calcd for $C_{24}H_{45}N_3O_7$: C, 59.17; H, 9.31; N, 8.62. Found: C, 59.84; H, 9.30; N, 8.52.

Acknowledgment. The financial support of MURST is gratefully acnowledged. We thank Prof. G. Lucente (Dipartimento di Studi Farmaceutici, Università "La Sapienza", Roma, Italy) for useful discussions.

Supporting Information Available: Tabulation of elemental analyses, mass spectra, ^{1}H and ^{13}C NMR data for substrates **1**, **3**, **5**, **7**, **10**, **12**−**14**, **16**, **17**, and **20** and ^{1}H, ^{13}C, and ^{13}C(INEPT) NMR spectra of **1**, **3**, **5**, **7**, **8**, **20**, and **21**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO990185W