New Oxazole-Based Conformationally Restricted Peptidomimetics: Design and Synthesis of Pseudopeptides

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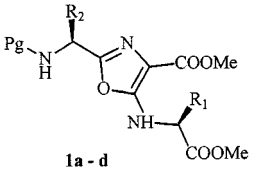
A general synthesis of a new class of optically active amino acids containing an oxazole moiety is described along with a strategy for their insertion into a peptidomimetic chain. A modified portion of endothelin-1 is prepared as a potential receptor antagonist. The procedure presented is based on readily available materials and can be performed in multigram quantities.

Introduction

The rational design of therapeutic agents that attenuate the pharmacological effects of peptides is one of the major goals of medicinal chemistry. For this reason, peptidomimetics have become very important for organic and medicinal chemistry. These peptidomimetics must be chemically and enzymatically stable and should possess acceptable bioavailability and pharmacokinetics. The design of peptidomimetics as potential bioactive substances may take particular account of the stabilization of the conformation by the introduction of elements conferring rigidity[1] or stabilizing secondary structures.[2] Several building blocks, able to specifically stabilize some parts of the peptide side chain or backbone, have been designed.[3]

In continuing our studies directed towards the preparation of unnatural amino acids,[4] we have already reported the synthesis of oxazole-containing amino acids.[5] Analogous compounds have been considered as precursors of peptidomimetic structures[6] and constituents of biologically active natural-like marine metabolites.[7]

During these studies we designed a general enantioconservative synthesis of the new polyfunctional oxazole amino acids **1**, which have at least two stereogenic centres (Scheme 1), and which may constitute an interesting example of dipeptide mimics. In fact, compounds **1** can be inserted into a peptide backbone following two different strategies: the pseudopeptide chain may be continued starting either from the carboxylic group bound to the 4-position of the oxazole ring, thus probably forming a turn mimetic, or from the amino acid residue in the 5-position of the heterocycle ring. This last sequence is comparable to the H−Xaa−Asp−Xaa−OH one.



	R_1	R_2	Pg
1a	*i*-Pr	CH_2Ph	Cbz
1b	*s*-Bu	CH_2COOBn	Boc
1c	CH_2Ph	Me	Cbz
1d	Me	*i*-Bu	Cbz

Scheme 1

Considering that the use of these oxazole amino acids as building blocks has already been checked, we have tested the possibility of assembling the oxazole moiety directly onto the peptide chain with a view also to extending the procedure to the solid phase. Therefore we have undertaken the preparation of a pseudopeptide, developed from the C-terminal hexapeptide His^{16}−Trp^{21} of the endothelin-1 receptor[8] (Scheme 2), by the replacement of the CO−N−C−CO network with a five-membered ring. The modification introduced in the structure would affect the preferred conformations of the peptide backbone and should influence the disposition of the adjacent residues. It is noteworthy that similar oxazole-containing pseudopeptides have already been found to bind to endothelin receptors.[9]

Results and Discussion

The preparation of compounds **1a−d** has been approached from a retrosynthetic correlation of the oxazole ring with a tripeptide that could be prepared from an *N*-protected α-amino acid amide by rhodium(II) catalyzed N−H insertion of an *N*-(1-methoxycarbonylalkyl)-2-diazo malonamic acid methyl ester, by modifying a previously reported procedure.[3] For this purpose, we prepared the 2-(2-methoxycarbonylacetylamino)alkyl acetic acid methyl esters **2**, which were then reacted with 4-acetamidobenzensulfonyl azide and Et_3N in benzene at room temperature to give the corresponding diazo derivatives **3**. Subsequent reaction with a $CHCl_3$ solution of the amide of the protected amino acid **4**, in the presence of Rh^{II} acetate dimer (catalytic

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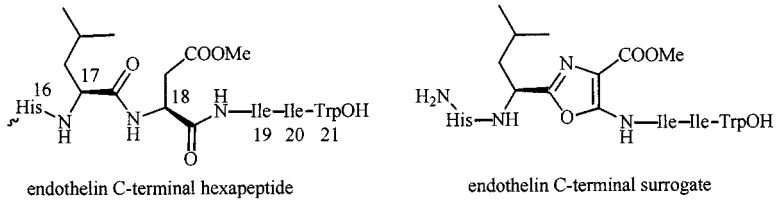
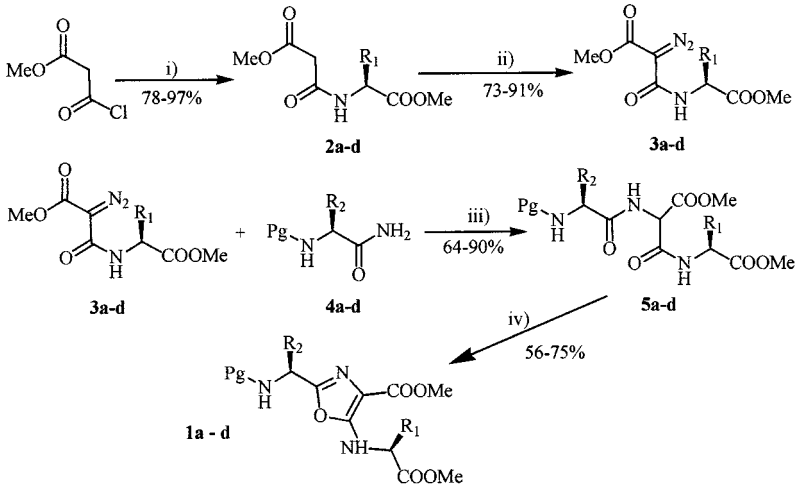


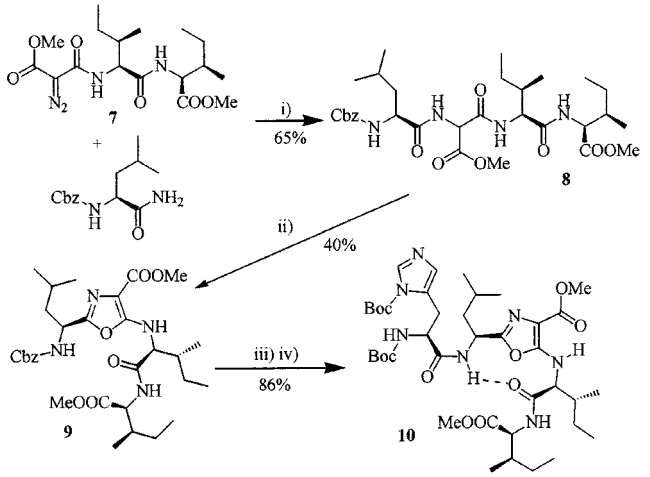
Scheme 2



Scheme 3. i) H−XXX−OMe, CH_2Cl_2, NMM (2 equiv.), −15 °C; ii) 4-acetamidobenzensulfonyl azide, Et_3N, benzene, room temp., 40 h; iii) $Rh_2(OAc)_4$ cat, $CHCl_3$, 65 °C; iv) PPh_3, I_2, Et_3N, CH_2Cl_2, room temp.

amount), furnished compounds **5** in good yield. Compounds **5** were then cyclodehydrated to the oxazole derivatives **1** with PPh_3 and I_2 in the presence of Et_3N in dichloromethane (Scheme 3).[10]

The synthetic protocol we have chosen for preparing the optically active oxazole-containing amino acids appeared to be convenient and versatile. Therefore, in order to confirm the applicability of the sequence scheme to more complex systems, we have undertaken the synthesis of the pseudopeptide fragment His[16]−Leu[17]−**1** (R = *i*Bu, R_1 = *s*Bu)−Ile[20] (**10**). We started by preparing the known dipeptide H−Ile−Ile−OMe, which was then reacted with chlorocarbonyl acetic acid methyl ester to give the corresponding tripeptide mimetic **6**. The subsequent reaction of **6** with *p*-toluenesulfonyl azide in benzene/Et_3N at room temperature gave the diazopeptide **7** (Scheme 4), which was purified by flash chromatography.



Scheme 4. i) $Rh_2(OAc)_4$ cat, $CHCl_3$, 65 °C; ii) PPh_3, I_2, Et_3N, CH_2Cl_2, room temp.; iii) 10% Pd/C, MeOH, cyclohexene, HCl (3N), reflux; iv) NMM, isobutyl chloroformate, di-Boc-histidine, CH_2Cl_2, −15 °C

When *N*-benzyloxycarbonyl L-leucinamide was treated with the diazo compound **7** in $CHCl_3$ in the presence of catalytic amount of Rh^{II} acetate dimer, the reaction proceeded smoothly to produce compound **8**, from which the oxazole derivative **9** was obtained in good yield. The oxazole peptide mimetic was then *N*-deprotected by hydrogenolysis of the benzyl carbamate in the presence of 10% Pd/C; the successive amide coupling with *di*-Boc-histidine[11] afforded the desired fragment **10** in good yield (86%).

The introduction of the heterocyclic ring in the peptidic framework decreases the conformational freedom of compound **10** and an intramolecular Leu(NH)···O=C(Ile) bond would be expected. To control the formation of this bond, the structure of **10** was analyzed by extensive ^1H NMR experiments. First of all, we needed to assign the resonances of the N−H protons with COSY experiments. Afterwards we observed a downfield shift of the Leu(NH) signal from δ = 8.04 (0.1 M solution in $CDCl_3$) to δ = 8.27 (0.01 M in $CDCl_3$). Moreover, almost invariant chemical shifts for the Leu(NH) and for the N−H proton in the 5-position of the oxazole ring are observed when **10** was dissolved in $CDCl_3$ with various concentrations of [D_6]DMSO. In addition, on varying the temperature from 25 °C to 50 °C, the above N−H signals exhibit temperature coefficients close to zero,

whereas the other N−H protons reveal a slight temperature dependence. All the collected data confirm that, at least in $CHCl_3$ solution, an intramolecular (Leu)N−H···O=C(Ile) bond occurs, as depicted in Scheme 4.

Conclusions

In summary, we have demonstrated the possibility of assembling oxazole-containing amino acids directly onto peptide chains. The synthesis of other heterocycle-based amino acids is currently under investigation as to their use as scaffolds[12] in combinatorial organic chemistry[13] both in solution and in the solid phase, taking into account the possibility of the orthogonal derivatizations presented by these compounds.

Experimental Section

General Procedures: Boiling points are uncorrected. Elemental analyses were performed on a Perkin−Elmer 420 B analyser. Optical rotations were measured with a Perkin−Elmer 241 automatic polarimeter in a 1 dm tube. The ^1H (300 MHz) and ^{13}C NMR (75.4 MHz) spectra were obtained with a Varian VXR-300 spectrometer on $CDCl_3$ solutions at 25° and 50 °C in order to show the eventual presence of conformers that affect ^1H and ^{13}C resonances at the lower temperature, causing considerable line broadening and duplication of signals in compounds **1**, **5**, **7**, **9**, **10**. All reactions involving air sensitive materials were carried out under N_2 atmosphere; all reagents and solvents employed were reagent grade materials purified by standard methods and distilled before use. The $CHCl_3$ was washed seven times with H_2O, dried over $CaCl_2$ overnight, filtered and stored over molecular sieves at 5 °C. As chiral starting material, (*S*)-α-amino acids of "*BioChemika*" grade (chemical and enantiomeric purity >99%) purchased from Fluka Chemie AG were used.

The amide **4** was prepared according to a previously described procedure.[3]

(*S*)-4-*tert*-Butoxycarbonyl asparagine benzyl ester **(4b)** and (*S*)-*N*-benzyloxycarbonyl phenyl alanylamide **(4a)** are known.[5]

(*S*)-*N*-Benzyloxycarbonyl Alanylamide (4c): TLC: EtOAc/CH_2Cl_2 9:2; 87% yield. − ^1H NMR: δ = 1.39 (d, *J* = 7.1 Hz, 3 H), 4.33−4.19 (m, 1 H), 5.10 (br. s, 2 H), 5.41 (d like, *J* = 7.2 Hz, 1 H, NH), 5.63 (br. s, 1 H, NH), 6.12 (br. s, 1 H, NH), 7.44−7.28 (m, 15 H). − ^{13}C NMR: δ = 16.8, 56.2, 69.6, 127.2, 127.4, 128.7, 140.9, 157.5, 177.2. − $C_{11}H_{14}N_2O_3$ (222.10): calcd. C 59.45, H 6.35, N 12.61; found C 59.41, H 6.29, N 12.68.

(*S*)-*N*-Benzyloxycarbonyl Leucinylamide (4d): TLC: EtOAc/petroleum ether 6:4; 94% yield. − ^1H NMR: δ = 0.93 (d, *J* = 6.0 Hz, 6 H), 1.58−1.43 (m, 1 H), 1.75−1.59 (m, 2 H), 4.27−4.14 (m, 1 H), 5.10 (br. s, 2 H), 5.22 (d like, *J* = 8.1 Hz, 1 H, NH), 5.57 (br. s, 1 H, NH), 6.10 (br. s, 1 H, NH), 7.42−7.28 (m, 5 H). − ^{13}C NMR: δ = 21.5, 22.6, 40.8, 56.9, 69.6, 127.2, 127.4, 128.7, 140.9, 157.5, 176.2. − $C_{14}H_{20}N_2O_3$ (264.15): calcd. C 63.62, H 7.63, N 10.60; found C 63.58, H 7.67, N 10.63.

General Procedure for the Preparation of the Malonamides 2: To the appropriate amino acid methyl ester (17.9 mmol) dissolved in dry CH_2Cl_2 (50 mL) at −15 °C under vigorous stirring were added slowly 4-methylmorpholine (4 mL, 35.8 mmol) and methylmalonyl chloride (1.92 mL, 17.9 mmol). The mixture was stirred at −15 °C for 20 min. After 12 h of stirring at room temp., the solvent was evaporated in vacuo. To the residue were added H_2O (20 mL) and EtOAc (20 mL) and the aqueous layer was discarded. The organic solution was washed with 10% aq. $KHSO_4$, sat. aq. NaCl, 10% aq. $NaHCO_3$, sat. aq. NaCl (30 mL), in that order, and dried (Na_2SO_4). The solvent was evaporated under reduced pressure affording pure malonamides **2** as described below.

(*S*)-2-(2-Methoxycarbonylacetylamino)-3-methylbutanoic Acid Methyl Ester (2a): TLC: EtOAc/petroleum ether 6:4; 90% yield. − ^1H NMR ($CDCl_3$): δ = 0.81−1.01 (m, 6 H), 2.09−2.27 (m, 1 H), 3.32−3.38 (m, 2 H), 3.72 (s, 3 H), 3.75 (s, 3 H), 4.54 (dd, J_1 = 4.9, J_2 = 8.6 Hz, 1 H), 7.55 (d, *J* = 7.74 Hz, 1 H, NH). − ^{13}C NMR: δ = 17.9, 19.2, 31.3, 41.2, 52.4, 52.7, 57.5, 165.0, 169.9,172.3. − $C_{10}H_{17}NO_5$ (231.11): calcd. C 51.94, H 7.41, N 6.06; found C 51.90, H 7.44, N 6.10.

(2*S*, 3*R*)-2-(2-Methoxycarbonylacetylamino)-3-methylpentanoic Acid Methyl Ester (2b): TLC: EtOAc/petroleum ether 4:6; 88% yield − ^1H NMR ($CDCl_3$): δ = 0.92−0.79 (m, 6 H), 1.46−1.04 (m, 2 H), 1.93−1.78 (m, 1 H), 3.30 (br. s, 2 H), 3.58 (s, 3 H), 3.66 (s, 3 H), 4.52 (dd, J_1 = 4.9, J_2 = 8.5 Hz, 1 H), 7.57 (d, *J* = 8.0 Hz, 1 H, NH). − ^{13}C NMR: δ = 11.1, 15.1, 24.7, 37.3, 41.0, 51.6, 51.9, 56.3, 165.2, 168.8, 171.7. − $C_{11}H_{19}NO_5$ (245.13): calcd. C 53.87, H 7.81, N 5.71; found C 53.90, H 7.76, N 5.73.

(*S*)-2-(2-Methoxycarbonylacetylamino)-3-phenylpropanoic Acid Methyl Ester (2c): TLC: EtOAc/ petroleum ether 1:1; 97% yield. − ^1H NMR ($CDCl_3$): δ = 3.26−3.04 (m, 2 H), 3.34 (br. s, 2 H), 3.82−3.67 (m, 6 H), 4.96−4.83 (m, 1 H), 7.59−7.10 (m, 6 H). − ^{13}C NMR: δ = 37.8, 41.3, 52.4, 52.5, 53.6, 127.2, 129.4, 135.9, 164.9, 169.2, 171.8. − $C_{14}H_{17}NO_5$ (279.11): calcd. C 60.21, H 6.14, N 5.02; found C 60.25, H 6.17, N 5.06.

(*S*)-2-(2-Methoxycarbonylacetylamino)-propanoic Acid Methyl Ester (2d): TLC: EtOAc/petroleum ether 6:4; 78% yield. − ^1H NMR ($CDCl_3$): δ = 1.47−1.37 (m, 3 H), 3.30 (br. s, 2 H), 3.76−3.67 (m, 6 H), 4.62−4.50 (m, 1 H), 7.57 (br. d, 1 H, NH). − ^{13}C NMR: δ = 18.1, 41.1, 48.2, 52.5, 165.1, 169.3, 173.1. − $C_8H_{13}NO_5$ (203.08): calcd. C 47.29, H 6.45, N, 6.89; found C 47.31, H 6.49, N 6.85.

General Procedure for the Preparation of 2-Diazo-*N*-(1-methoxycarbonylalkyl)malonamic Acid Methyl Esters (3): A solution of 4-acetamidobenzensulfonyl azide (12.4 mmol), Et_3N (12.9 mmol), and malonamides **4** (12.9 mmol) in dry benzene (20 mL) was allowed to stand at room temp. for 2 h, after which time a solid precipitated. After standing for 48 h at room temp., the solvent was removed under reduced pressure and the residue was separated from the by-products by flash chromatography on silica gel to afford the pure diazomalonamides.

(2*S*,3*S*)-2-(2-Methoxycarbonyl-2-diazoacetylamino)-3-methylbutanoic Acid Methyl Ester (3a): TLC: EtOAc/petroleum ether 3:7; 91% yield. − ^1H NMR ($CDCl_3$): δ = 0.94 (dd, J_1 = 6.9, J_2 = 10.2 Hz, 6 H), 2.11−2.32 (m, 1 H), 3.71 (s, 3 H), 3.84 (s, 3 H), 4.46−4.37 (m, 1 H), 4.56 (dd, J_1 = 4.7, J_2 = 8.5 Hz, 1 H). Mixture of conformers: ^{13}C NMR: δ = 17.6, 19.1, 31.0, 41.3, 52.1, 52.5, 57.6, 160.6, 164.7, 171.9. − $C_{10}H_{15}N_3O_5$ (257.10): calcd. C 46.69, H 5.88, N 16.33; found C 46.71, H 5.90, N 16.30.

(*S*)-2-(2-Methoxycarbonyl-2-diazoacetylamino)-3-methylpentanoic Acid Methyl Ester (3b): TLC: EtOAc/petroleum ether 3:7; 83% yield. − ^1H NMR ($CDCl_3$): δ = 0.86−0.68 (m, 6 H), 1.41−0.97 (m, 2 H), 1.91−1.78 (m, 1 H), 3.73 (s, 3 H), 3.50 (s, 3 H), 4.48 (dd,

J_1 = 4.8, J_2 = 8.5 Hz, 1 H), 8.02 (s, 1 H, NH). − ^{13}C NMR: δ = 11.6, 15.7, 25.1, 37.7, 52.1, 52.5, 57.1, 160.6, 164.7, 171.9. − $C_{11}H_{17}N_3O_5$ (271.12): calcd. C 48.70, H 6.32, N 15.49; found C 48.68, H 6.29, N 15.52.

(*S*)-2-(2-Methoxycarbonyl-2-diazoacetylamino)-3-phenylpropanoic Acid Methyl Ester (3c): TLC: EtOAc/petroleum ether 4:6; 80% yield. − ^{1}H NMR ($CDCl_3$): δ = 3.17 (dd, J_1 = 7.1, J_2 = 13.9 Hz, 1 H), 3.27 (dd, J_1 = 5.6, J_2 = 19.3 Hz, 1 H), 3.78 (s-like, 3 H), 3.88 (s-like, 3 H), 4.98 (dd, J_1 = 6.0, J_2 = 13.3 Hz, 1 H), 7.50−7.04 (m, 5 H), 8.15 (br. s, 1 H, NH). − ^{13}C NMR: δ = 38.1, 52.4, 52.5, 53.7, 54.0, 127.2, 128.7, 129.2, 135.9, 160.4, 164.3, 171.6. − $C_{14}H_{15}N_3O_5$ (305.10): calcd. C 55.08, H 4.95, N 13.76; found C 55.10, H 4.98, N 13.72.

(*S*)-2-(2-Methoxycarbonyl-2-diazoacetylamino)-propanoic Acid Methyl Ester (3d): TLC: EtOAc/petroleum ether 4:6; 73% yield. − ^{1}H NMR ($CDCl_3$): δ = 1.47−1.36 (m, 3 H), 3.75−3.68 (m, 3 H), 3.84−3.78 (m, 3 H), 4.67−4.53 (m, 1 H), 8.06 (br. s, 1 H, NH). − ^{13}C NMR: δ = 18.2, 48.1, 48.4, 52.4, 160.2, 164.4, 172.8. − $C_8H_{11}N_3O_5$ (229.07): calcd. C 41.92, H 4.84, N 18.33; found C 41.89, H 4.80, N 18.35.

General Procedure for the Preparation of Compounds 5: A solution of the diazomalonamides **3** (6.16 mmol) in dry chloroform (30 mL) was added dropwise over 3 h to a refluxing solution of the proper amide (4.4 mmol) and $Rh_2(OAc)_4$ (44 mg, 0.1 mmol) in dry chloroform (114 mL). The mixture was refluxed for a further 24 h, allowed to cool, evaporated in vacuo and the crude product purified by flash chromatography on silica to give the products **5** described below.

(*S*)-2-[2-(*S*)-(2-Benzyloxycarbonylamino-3-phenylpropanoylamino)-2-methoxycarbonylacetylamino]-3-methylbutanoic Acid Methyl Ester (5a): TLC: EtOAc/petroleum ether 6:4; 83% yield. − ^{1}H NMR ($CDCl_3$) mixture of conformers: δ = 1.00−0.64 (m, 6 H), 2.26−1.99 (m, 1 H), 3.27−2.75 (m, 2 H), 3.79−3.46 (m, 7 H), 4.61−4.44 (m, 1 H), 5.08−4.79 (m, 2 H), 5.49 (d, J = 7.6 Hz, 0.6 H), 5.59 (d, J = 7.6 Hz, 0.4 H), 6.44−6.25 (m, 1 H, NH), 7.39−6.96 (m, 10 H), 7.85 (br. s, 1 H, NH), 7.99 (d, J = 7.2 Hz, 0.6 H, NH), 8.11 (d, J = 7.3 Hz, 0.4 H, NH). − ^{13}C NMR: δ = 17.7, 18.9, 31.5, 38.8, 52.1, 52.9, 53.1, 55.6, 57.7, 66.6, 126.6, 127.7, 127.9, 128.2, 128.3, 129.5, 136.5, 136.7, 156.2, 165.2, 167.5, 171.7, 172.1. − $C_{27}H_{33}N_3O_8$ (527.23): calcd. C 61.47, H 6.30, N 7.96; found C 61.50, H 6.28, N 7.92.

(2*S*,3*R*)-2-[2-(*S*)-(3-Benzyloxycarbonyl-2-tert-butoxycarbonylaminopropanoylamino)-2-methoxy-carbonylacetylamino]-3-methylpentanoic Acid Methyl Ester (5b): TLC: EtOAc/petroleum ether 1:1; 90% yield. − ^{1}H NMR ($CDCl_3$): δ = 0.99−0.83 (m, 8 H), 1.45 (s, 9 H), 2.00−1.83 (m, 1 H), 3.19−2.70 (m, 2 H), 3.86−3.69 (m, 6 H), 4.72−4.69 (m, 2 H), 5.2−5.0 (m, 3 H), 5.6 (d, J = 8.5 Hz, 1 H, NH), 7.0−6.8 (m, 1 H, NH), 7.4−7.3 (m, 5 H), 7.7−7.5 (m, 1 H, NH). − ^{13}C NMR: δ = 11.9, 15.8, 17.2, 25.4, 28.7, 30.1, 38.6, 52.6, 53.7, 57.4, 62.0, 67.2, 128.4, 128.7, 128.9, 135.7, 164.4, 167.7, 171.1, 171.3, 171.7, 171.9. − $C_{27}H_{39}N_3O_{10}$ (565.26): calcd. C 57.33, H 6.95, N 7.43; found C 57.36, H 6.97, N 7.39.

2-[(*S*)-2-Benzyloxycarbonylaminopropanoylamino]-*N*-[(*S*)-1-methoxycarbonyl-2-phenyleth-yl]-malonamic Acid Methyl Ester (5c): TLC: EtOAc/petroleum ether 6:4; 75% yield. − ^{1}H NMR ($CDCl_3$): δ = 1.48−1.13 (m, 3 H), 3.24−2.99 (m, 2 H), 3.84−3.56 (m, 7 H), 4.4−4.05 (m, 1 H), 4.65 (s, 1 H, NH), 4.83 (s, 1 H), 5.10 (s, 2 H), 5.59 (br. s, 1 H, NH), 7.62−6.85 (m, 11 H). − ^{13}C NMR: δ = 19.3, 37.8, 50.1, 53.3, 53.7, 55.1, 57.2, 66.6, 127.8, 129.0, 129.1, 129.5, 130.3, 138.1, 138.3, 156.9, 166.1, 168.9, 172.7, 174.4. − $C_{25}H_{29}N_3O_8$ (499.20): calcd. C 60.11, H 5.85, N, 8.41; found C 60.15, H 5.82, N 8.38.

2-[(*S*)-2-Benzyloxycarbonylamino-4-methylpentanoylamino]-*N*-[(*S*)-1-methoxycarbonyleth-yl]-malonamic Acid Methyl Ester (5d): TLC: EtOAc/petroleum ether 6:4; 64% yield. − ^{1}H NMR ($CDCl_3$): δ = 1.01−0.77 (m, 6 H), 1.78−1.28 (m, 6 H), 3.84−3.63 (m, 7 H), 4.40−4.24 (m, 1 H), 4.61−4.46 (m, 1 H), 5.23−5.01 (m, 3 H), 5.51−5.31 (m, 1 H, NH), 7.49−6.99 (m, 6 H). − ^{13}C NMR: δ = 17.2, 18.4, 23.2, 24.9, 48.9, 52.9, 53.7, 56.8; 61.9, 67.4, 128.3, 128.5, 128.8, 136.4, 156.5, 164.5, 167.7, 172.8, 172.9. − $C_{22}H_{31}N_3O_8$ (465.21): calcd. C 56.76, H 6.71, N 9.03; found C 56.78, H 6.69, N 9.10.

General Procedure for the Preparation of the Oxazole Derivatives 1: To a stirred solution of PPh_3 (1.6 g, 6 mmol) and I_2 (1.5 g, 6 mmol) in dry dichloromethane (40 mL) at room temp. were added sequentially Et_3N (1.7 mL, 12 mmol) and compound **5** (3 mmol), in dry dichloromethane (10 mL). The mixture was stirred overnight, the solvents evaporated in vacuo, and the crude product purified by flash chromatography on silica gel to give the oxazole derivatives **1**.

2-[(*S*)-1-Benzyloxycarbonylamino-2-phenylethyl]-5-[(*S*)-1-methoxycarbonyl-2-methylprop-ylamino]-oxazole-4-carboxylic Acid Methyl Ester (1a): TLC: EtOAc/petroleum ether 3:7; 56% yield. − $[\alpha]_D^{18}$ = −33.5 (c =1.21, CH_2Cl_2). − ^{1}H NMR ($CDCl_3$:) δ = 0.88−0.80 (m, 6 H), 2.12−1.95 (m, 1 H), 3.11 (AB system, J = 6.8 Hz, 2 H), 3.80 (s, 3 H), 3.57 (s, 3 H), 4.02−3.91 (m, 1 H), 5.08−4.92 (m, 3 H), 6.48 (d, J = 9.1 Hz, 1 H, NH), 7.34−6.93 (m, 11 H). − ^{13}C NMR: δ = 17.8, 19.1, 31.6, 40.5, 50.5, 52.6, 58.1, 61.4, 67.0, 127.1, 128.2, 128.5, 128.7, 129.3, 129.4, 135.8, 136.3, 151.7, 155.6, 159.4, 163.9, 171.4, 174.1. − $C_{27}H_{31}N_3O_7$ (509.22): calcd. C 63.64, H 6.13, N 8.25; found C 63.70, H 6.11, N 8.21.

2-[(*S*)-2-Benzyloxycarbonyl-1-*tert*-butoxycarbonylaminoethyl)-5-[(1*S*,2*R*)1-methoxycarbon-yl-2-methylbutylamino]-oxazole-4-carboxylic Acid Methyl Ester (1b): TLC: EtOAc/petroleum ether 3:7; 66% yield. − $[\alpha]_D^{18}$ = −10.4 (c =1.32, CH_2Cl_2). − ^{1}H NMR ($CDCl_3$) mixture of conformers: δ = 0.95−0.83 (m, 8 H), 1.42 (br. s, 9 H), 1.98−1.73 (m, 2 H), 3.04−2.95 (m, 1 H), 3.71 (br. s, 3 H), 3.74 (br. s, 3 H), 4.20 (dd, J_1 = 5.0 Hz, J_2 = 9.3 Hz, 1 H), 4.44 (dd, J_1 = 5.0 Hz, J_2 = 8.8 Hz, 1 H), 5.44 (d, J= 8.9 Hz, 0.4 H, NH), 5.08 (s, 2 H), 5.55 (d, J = 9.1 Hz, 0.6 H, NH), 6.55 (d, J = 9.1 Hz, 1 H, NH), 7.38−7.24 (m, 5 H). − ^{13}C NMR: δ = 11.3, 15.3, 24.6, 25.0, 28.1, 29.5, 37.5, 38.1, 45.1, 57.1, 60.4, 66.5, 127.9, 128.1, 128.4, 135.2, 150.6, 154.7, 157.1, 159.4, 163.7, 169.9, 173.7. − $C_{27}H_{37}N_3O_9$ (547.25): calcd. C 59.22, H, 6.81, N 7.67; found C 59.25, H 6.79, N 7.65.

2-[(*S*)-1-Benzyloxycarbonylaminoethyl]-5-[(*S*)-1-methoxycarbonyl-2-phenylethylamino]-ox-azole-4-carboxylic Acid Methyl Ester (1c): TLC: EtOAc/petroleum ether 6:4; 75% yield. − $[\alpha]_D^{20}$ = −28.0 (c = 1.48, CH_2Cl_2). − ^{1}H NMR ($CDCl_3$) mixture of conformers: δ = 1.47−1.14 (m, 3 H), 3.27−2.98 (m, 2 H), 3.82−3.60 (m, 7 H), 4 91−4.65 (m, 1 H), 5.15−4.95 (m, 2 H), 5.36 (d, J = 9.7 Hz, 0.4 H, NH), 6.5 (d, J = 9.2 Hz, 0.36 H, NH), 7.73−6.91 (m, 11 H). − ^{13}C NMR: δ = 19.9, 37.5, 51.5, 51.8, 53.5, 67.1, 127.3, 127.5, 128.1, 128.4, 128.8, 129.6, 135.6, 136.3, 136.6, 152.8, 159.0, 163.8, 171.2. − $C_{25}H_{27}N_3O_7$ (481.18): calcd. C 62.36, H, 5.65, N 8.73; found C 62.40, H 5.62, N 8.69.

2-[(*S*)-1-Benzyloxycarbonylamino-3-methylbutyl]-5-[(*S*)-1-methoxycarbonylethylamino]-oxazole-4-carboxylic Acid Methyl Ester (1d): TLC: EtOAc/petroleum ether 1:1; 72% yield. − $[\alpha]_D^{20}$ = −40.6 (c = 1.43, CH_2Cl_2). − ^{1}H NMR ($CDCl_3$) mixture of conformers: δ = 0.93−0.78 (m, 6 H), 1.73−1.30 (m, 6 H), 3.84−3.66 (m, 6 H),

4.42−4.19 (m, 1 H), 4.94−4.77 (m, 1 H), 5.14−4.98 (m, 2 H), 5.23 (d, J = 8.5 Hz, 0.5 H, NH), 6.49 (d, J = 7.9 Hz, 0.5 H, NH), 7.50−7.16 (m, 6 H). − ^{13}C NMR: δ = 18.8, 21.8, 22.4, 24.4, 29.4, 42.8, 47.2, 51.2, 52.6, 66.8, 127.8, 127.9, 128.3, 135.9, 152.6, 155.4, 158.6, 163.6, 172.2, 174.3. − $C_{22}H_{29}N_3O_7$ (447.20): calcd. C 59.05, H, 6.53, N 9.39; found C 59.10, H 6.56, N 9.41.

Preparation of 2-[2-(2-Diazo-2-methoxycarbonylacetylamino)-3-methylpentanoylamino]-3-methylpentanoic Acid Methyl Ester (7): The preparation of compound **7** was achieved following the general procedure reported for compounds **3**, starting from pure 2-[2-(2-methoxycarbonylacetylamino)-3-methyl-pentanoylamino]-3-methylpentanoic acid methyl ester (**6**) and with p-TsN_3 instead of 4-acetamidobenzensolfonylazide:[14] TLC: EtOAc/petroleum ether 4:6; 52% yield. − ^{1}H NMR mixture of conformers: δ = 1.68−0.60 (m, 16 H), 2.04−1.71 (m, 2 H), 3.94−3.45 (m, 6 H), 4.66−4.15 (m, 2 H), 6.73 (d, J = 8.2 Hz, 1 H, NH), 8.09 (br. s, 1 H, NH). − ^{13}C NMR: δ = 11.2, 11.4, 15.3, 15.5, 24.6, 25.1, 37.1, 37.5, 51.9, 52.4, 57.0, 58.1, 160.6, 164.3, 170.6, 172.0, 173.2. − $C_{17}H_{28}N_4O_6$ (384.20): calcd. C 53.11, H 7.34, N 14.57; found C 53.14, H 7.39, N 14.61.

2-{2-[2-(2-Benzyloxycarbonylamino-4-methylpentanoylamino)-2-methoxycarbonylacetyl-amino]-3-methylpentanoylamino}-3-methylpentanoic Acid Methyl Ester (8): TLC: EtOAc/CH_2Cl_2 3:7; 44% yield. − ^{1}H NMR mixture of diastereoisomers: δ = 1.00−0.71 (m, 18 H), 1.22−1.00 (m, 3 H), 1.78−1.28 (m, 6 H), 3.79−3.50 (m, 6 H), 4.67−4.31 (m, 3 H), 5.18−4.91 (m, 2 H), 5.42−5.19 (m, 1 H), 5.73 (br. d, 1 H, NH), 6.89 (d, J = 7.9 Hz, 0.5 H, NH), 7.13 (br. d, 0.5 H, NH), 7.36−7.19 (m, 5 H), 7.54 (br. s, 1 H, NH), 7.93 (d, J = 6.5 Hz, 1 H, NH). − ^{13}C NMR: δ = 11.5, 11.8, 15.1, 15.5, 22.6, 23.1, 24.6, 24.8, 25.1, 25.2, 37.9, 38.0, 52.2, 52.4, 52.9, 56.4, 56.6, 66.7, 66.8, 128.1, 128.5, 128.7, 136.9, 156.4, 165.7, 167.8, 171.1, 172.8, 173.6. − $C_{31}H_{48}N_4O_9$ (620.35): calcd. C 59.98, H 7.79, N 9.03; found C 59.94, H 7.82, N 9.07.

2-(1-Benzyloxycarbonylamino-3-methylbutyl)-5-[1-(1-methoxycarbonyl-2-methylbutylcar-bamoyl)-2-methylbutylamino]-oxazole-4-carboxylic Acid Methyl Ester (9): TLC: EtOAc/petroleum ether 4:6; 38% yield. − $[\alpha]_D^{16}$ = −13.8 (c =1.48, CH_2Cl_2). − ^{1}H NMR mixture of conformers: δ = 2.04−0.57 (m, 27 H), 2.74−2.45 (m, 1 H), 3.92−3.48 (m, 6 H), 4.35−4.18 (m, 1 H), 4.65−4.44 (m, 1 H), 5.35−4.80 (m, 3 H), 6.55−6.36 (m, 1 H, NH), 7.83−7.18 (m, 6 H). − ^{13}C NMR: δ = 11.6, 11.9, 15.8, 16.0, 22.9, 24.6, 24.8, 25.4, 29.9, 32.8, 37.5, 37.8, 43.2, 52.1, 52.4, 56.7, 62.8, 67.2, 67.5, 128.1, 128.4, 128.7, 129.0, 131.1, 136.4, 156.1, 159.7, 169.0, 170.5, 172.4. − $C_{31}H_{46}N_4O_8$ (602.34): calcd. C 61.78, H 7.69, N 9.30; found C 61.69, H 7.63, N 9.38.

Preparation of N^{α}-,N^{π}-Di-*tert*-butyloxycarbonyl-L-histidine: Triethylamine (0.68 mL, 5.16 mmol) and then di-*tert*-butyl pyrocarbonate (1.24 g, 5.68 mmol) were added to a suspension of L-histidine (0.4 g, 2.58 mmol) in methanol (10 mL) and the mixture stored at room temp. overnight. The solvent was then eliminated in vacuo. To the residue were added H_2O (20 mL) and diethyl ether (50 mL) and the aqueous layer was separated. The aqueous solution was acidified to pH 2−3 with a 20% solution of $KHSO_4$ then extracted with EtOAc (3 × 10 mL). The combined organic solutions were dried over Na_2SO_4. The solvent was evaporated under reduced pressure to afford the pure product (TLC: EtOAc/MeOH 7:3; 0.5 g, 55% yield). − ^{1}H NMR: δ = 1.42 (s, 9 H), 1.55 (s, 9 H), 3.31−3.04 (m, 2 H), 4.54−4.37 (m, 1 H), 5.39 (d, J = 6.32 Hz, 1 H, NH), 7.18 (s, 1 H), 7.67 (s, 1 H).

2-{1-[2-*tert*-Butoxycarbonylamino-3-(1-*tert*-butoxycarbonyl-1*H*-imidazol-4-yl)-propionylamino]-3-methylbutyl}-5-[1-(1-methoxycarbonyl-2-methylbutylcarbamoyl)-2-methylbutylamino]oxazole-4-carboxylic Acid Methyl Ester: Compound **9** (0.18 g, 0.298 mmol) was suspended in MeOH (25 mL) with 10% Pd on active charcoal (0.2 g) and cyclohexene (1.2 mL), in the presence of 1 equiv. of HCl. The reaction mixture was refluxed for 3 h. The resulting mixture was filtered on a celite pad and all the volatile products were eliminated under reduced pressure. The crude solid recovered was suspended in absolute MeOH (25 mL) and filtered again. The collected **9**·HCl was used in the next step without any further purification.

Isobutyl chloroformate (0.034 mL, 0.278 mmol) in CH_2Cl_2 (2 mL) at −15 °C was added slowly and with vigorous stirring to a solution of di-*tert*-butyloxycarbonyl-L-histidine (84 mg, 0.238 mL) and 4-methylmorpholine (0.030 mL, 0.277 mmol) in CH_2Cl_2 (2 mL).The mixture was stirred at −15 °C for 20 min, then crude **9**·HCl (0.12 g, 0.238 mL) was added. After 12 h stirring at room temp., the solvent was removed in vacuo. To the residue were added H_2O (10 mL) and EtOAc (10 mL) and the aqueous layer was discarded. The organic solution was washed with 10% aq. $KHSO_4$, sat aq. NaCl, 10% aq. $NaHCO_3$, sat. aq. NaCl (10 mL), in that order, and dried (Na_2SO_4). The solvent was evaporated under reduced pressure affording pure pseudopeptide **10**. − TLC: EtOAc/MeOH 7:3; 84% yield. − ^{1}H NMR (0.1 M, $CDCl_3$, 25 °C)[15] mixture of conformers: δ = 0.99−0.78 (m, 18 H), 1.29 (m, 4 H), 1.41 (s, 9 H), 1.57 (s, 9 H), 1.91 (m, 2 H), 2.03 (m, 1 H), 2.33 (m, 1 H), 2.84 (m, 1 H), 2.93 (m, 3 H), 3.03 (m, 2 H), 3.58 (s, 3 H), 3.85 (s, 3 H), 4.59−4.23 (m, 3 H), 6.80 (br. s, 1 H, NH), 7.15 (s, 1 H), 7.28 (br. s, 1 H, NH), 7.50 (br. s, 1 H, NH), 7.67 (s, 1 H), 8.04 (br. s, 1 H, NH). − ^{13}C NMR: δ = 11.1, 11.8, 14.0, 14.4, 19.3, 19.5, 23.0, 24.0, 25.8, 28.1, 28.5, 33.0, 34.2, 52.3, 52.5, 53.7, 56.8, 56.9, 57.4, 62.0, 65.8, 129.1, 131.2, 132.5, 137.0, 138.9, 147.0, 168.0, 169.4, 171.2, 171.7, 172.3, 173.4. − $C_{39}H_{63}N_7O_{11}$ (805.46): calcd. C 58.12, H 7.88, N 12.17; found C 58.16, H 7.65, N 12.18.

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[14] Prepared from 2-(2-amino-3-methylpentanoylamino)-3-methylpentanoic acid methyl ester hydrochloride and methylmalonyl chloride: TLC: EtOAc/petroleum ether 4:6; 94% yield. − ^{1}H NMR (mixture of conformers): δ = 1.49−0.53 (m, 16 H), 1.86−1.54 (m, 2 H), 3.24−3.18 (m, 2 H), 3.60−3.54 (m, 6 H), 4.51−4.22 (m, 2 H), 6.87 (d, *J* = 8.3 Hz, 0.5 H, NH), 7.63−7.41 (m, 1.5 H, NH). − ^{13}C NMR: δ = 11.1, 11.4, 15.2, 15.4, 24.7, 25.0, 37.1, 37.6, 41.3, 51.9, 52.3, 56.5, 57.8, 165.1, 169.6, 170.9, 172.0. − $C_{17}H_{30}N_2O_6$ (358.21): calcd. C 56.97, H 8.44, N 7.82; found C 56.92, H 8.39, N 7.86.

[15] ^{1}H NMR (0.01 M, $CDCl_3$, 25 °C): δ = 6.81 (br. s, 1 H, NH), 7.14 (s, 1 H), 7.30 (br. s, 1 H, NH), 7.58 (br. s, 1 H, NH), 7.66 (s, 1 H), 8.27 (br. s, 1 H, NH); (0.1 M, $CDCl_3$, 50 °C) δ = 6.72 (br. s, 1 H, NH), 7.14 (s, 1 H), 7.20 (br. s, 1 H, NH), 7.50 (br. s, 1 H, NH), 7.67 (s, 1 H), 8.03 (br. s, 1 H, NH); (0.01 M, $CDCl_3$, 50 °C): δ = 6.73 (br. s, 1 H, NH), 7.14 (s, 1 H), 7.22 (br. s, 1 H, NH), 7.56 (br. s, 1 H, NH), 7.66 (s, 1 H), 8.26 (br. s, 1 H, NH).

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