The invention relates to a compound having formula (I) wherein $R^a$ is -NR$^2R^2$ and $R^b$ is $R^3$, or $R^3$ is $R^1$ and $R^b$ is -NH-(SO$_2$)$_2$-m-R$^3$, m being 0 or 1; wherein $R^1$ is -(SO$_2$)$_m$-(1-6C)alkylene-COOH or an ester derivative thereof, n being 0 or 1; and m and n are not 1 at the same time; $R^2$ is selected from H, (1-12C)alkyl, (2-12C)alkenyl, (3-8C)cycloalkyl, (1-6C)alkylene(3-8C)cycloalkyl, (6-14C)aryalkyl, (7-15C)caryl and -(1-6C)alkylene-COOH or an ester derivative thereof; $R^3$ is a hydrophobic moiety; or $R^2$ and $R^3$ are a 5- or 6-membered ring together with "N-C" to which they are bound, which ring may be fused with an aliphatic or aromatic 6-membered ring; A is an amino acid selected from proline, optionally containing a second heteroatom selected from N, O, or S, and optionally substituted with (1-6C)alkyl, (1-6C)alkoxy or halogen, 3,4-dehydroproline, 2-azetidine carboxylic acid, piperelic acid, octahydroindole-2-carboxylic acid, 2-amino isobutyric acid or valine; B is lysine or 4-aminocyclohexylglycine; and X is -CHF$_2$, -CF$_3$, -COOR$^4$ or $-CONR^5R^6$, wherein $R^4$ is H or (2-6C)alkyl, and $R^5$ and $R^6$ are independently H, (1-6C)alkyl or -(1-6C)alkylene-C$_2$H$_5$, or $R^5$ and $R^6$ together are (3-6C)alkylene, or $X$ is a heterocycle selected from 2-thiazole, 2-thiazoline, 2-benzothiazole, 2-oxazole, 2-oxazolone and 2-benzimidazole, which heterocycles are optionally substituted with one or more (1-6C)alkyl, (1-6C)alkoxy or oxo; or a pharmaceutically acceptable salt thereof. The compounds of the invention have anticoagulant activity and can be used in treating or preventing thrombin-related diseases.
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THROMBIN INHIBITORS

The invention relates to thrombin inhibitors, pharmaceutical compositions containing the same, as well as the use of said inhibitors for treating and preventing thrombin-related diseases.

Thrombin is a member of the class of the serine proteases. Serine proteases are enzymes which, amongst other things, play an important role in the blood coagulation cascade. Other members of this class of proteases are for example trypsin, factors VIIa, IXa, Xa, XIa, XIIa, and protein C.

Thrombin is the serine protease which regulates the last step in the coagulation cascade. The prime function of thrombin is the cleavage of fibrinogen to generate fibrin monomers, which form an insoluble gel by cross-linking. In addition, thrombin regulates its own production by activating factors V and VIII earlier in the cascade. It also has important actions at cellular level, where it acts on specific receptors to cause platelet aggregation, endothelial cell activation and fibroblast proliferation. Thus thrombin has a central regulatory role in haemostasis and thrombus formation. Since inhibitors of thrombin may have a wide range of therapeutic applications, extensive research has been performed in this area.

In the development of synthetic inhibitors of serine proteases, and more specifically of thrombin, the interest in small synthetic peptides, that are recognized by proteolytic enzymes in a manner similar to that of natural substrates, has increased. As a result, new peptide-like inhibitors have been prepared, such as the transition state inhibitors of thrombin.

The search for more effective and more selective thrombin inhibitors continues unabated in order to obtain thrombin inhibitors which can be administered in lower dosages and which have fewer and less severe side effects.

Furthermore, special attention is paid to oral bioavailability. Potent intravenous thrombin inhibitors are clinically effective in acute care settings requiring the treatment of thrombin-related diseases. However, particularly the prevention of thrombin-related diseases such as myocardial infarct, thrombosis and stroke require long-term therapy, preferably by orally dosing of an anticoagulant.

Many of the peptide-like thrombin inhibitors disclosed in prior publications are based on the sequence -D-Phe-Pro-Arg-, see for example compounds as disclosed by Brady et al. [Bioorganic & Medical Chemistry, 3 (1995), 1063-78]. Thrombin inhibitors may also contain
lysine side chains instead of arginine, such as other inhibitors disclosed by Brady et al., and further by Jones et al. [J. Enzyme Inhibition, 2 (1995), 43-60] and Lewis et al. [Thrombosis and Haemostasis, 74(4) (1995), 1107-12]. Also thrombin inhibitors having an aminocyclohexyl moiety instead of lysine or arginine side chain are known [WO 94/25051]. From these and also other references [e.g. US 5,523,308] further a number of variations at the C-terminus of these peptide-like thrombin inhibitors is known. The developments in this field have already improved the understanding of how to modulate the biological properties of this type of thrombin inhibitors. However, although great effort is being spend on finding selective thrombin inhibitors having good oral bioavailability, there are still few transition state thrombin inhibitors known in the art which fulfill these requirements.

It has now been found that compounds having the formula 1

\[ R^a-CH-C(O)-A-B-X \]

\[ R^b \]

wherein \( R^a \) is \(-NR^1R^2\) and \( R^b \) is \( R^3 \), or \( R^3 \) is \( R^1 \) and \( R^b \) is \(-NH-(SO_2)_m-R^3 \), \( m \) being 0 or 1;

wherein \( R^1 \) is \(-(SO_2)_n-(1-6)alkylene-COOH\) or an ester derivative thereof, \( n \) being 0 or 1; and \( m \) and \( n \) are not 1 at the same time; \( R^3 \) is selected from \( H \), \( (1-12)alkyl \), \( (2-12)alkenyl \), \( (3-8)cycloalkyl \), \( (1-6)alkylene(3-8)cycloalkyl \), \( (6-14)aryl \), \( (7-15)alkyl \) and \(-(1-6)alkylene-COOH\) or an ester derivative thereof, \( R^3 \) is a hydrophobic moiety; or \( R^3 \) and \( R^3 \) are a 5- or 6-membered ring together with "N-C" to which they are bound, which ring may be fused with an aliphatic or aromatic 6-membered ring; \( A \) is an amino acid selected from proline, optionally containing a second heteroatom selected from \( N \), \( O \), or \( S \), and optionally substituted with \((1-6)alkyl\), \((1-6)alkoxy\) or \( halogen \), \( 3,4\)-dehydroproline, 2-azetidine carboxylic acid, piperolic acid, octahydroindole-2-carboxylic acid, 2-amino isobutyric acid or valine; \( B \) is lysine or 4-aminocyclohexylglycine, and \( X \) is \(-CHF_2\), \(-CF_3\), \(-COOR^4\) or \(-CONR^5R^6\), wherein \( R^4 \) is \( H \) or \((2-6)alkyl\), and \( R^5 \) and \( R^6 \) are independently \( H \), \((1-6)alkyl\) or \(-(1-6)alkylene-C_xH_y\), or \( R^5 \) and \( R^6 \) together are \((3-6)alkylene\), or \( X \) is a heterocycle selected from 2-thiazole, 2-thiazoline, 2-benzothiazole, 2-oxazole, 2-oxazoline, 2-benzoxazole and 2-benzimidazole, which heterocycles are optionally substituted with one or more \((1-6)alkyl\), \((1-6)alkoxy\) or \( oxo \), or a pharmaceutically acceptable salt thereof, are
potent and selective thrombin inhibitors which show good bioavailability after oral administration.

The compounds of the present invention are useful for treating and preventing thrombin-mediated and thrombin-associated diseases. This includes a number of thrombotic and prothrombotic states in which the coagulation cascade is activated which include, but are not limited to, deep vein thrombosis, pulmonary embolism, thrombophlebitis, arterial occlusion from thrombosis or embolism, arterial reocclusion during or after angioplasty or thrombolysis, restenosis following arterial injury or invasive cardiological procedures, postoperative venous thrombosis or embolism, acute or chronic atherosclerosis, stroke, myocardial infarction, cancer and metastasis, and neurodegenerative diseases. The compounds of the invention may also be used as anticoagulants in extracorporeal blood circuits, as necessary in dialysis and surgery.

The compounds of the invention may also be used as in vitro anticoagulants.

Preferred compounds according to the invention have the formula I, wherein $R^2$ is $H$, (1-12C)alkyl, (2-12C)alkenyl, benzyl or -(1-6C)alkylene-COOH or an ester derivative thereof; $R^3$ is (1-12C)alkyl, (3-8C)cycloalkyl, di(3-8C)cycloalkylmethyl, -(1-6C)alkylene-(3-8C)cycloalkyl, (6-14C)aryl, di(6-14C)aryl methyl, or -(1-6C)alkylene-(6-14C)aryl, wherein the cycloalkyl and aryl groups may optionally be substituted with one or more substituents selected from halogen, hydroxy, trifluoromethyl, (1-6C)alkyl or (1-6C)alkoxy, A is proline, 3,3-dimethylproline, thiazolidine-4-carboxylic acid, 4-cis-ethylproline, 3,4-dehydroproline, 2-azetidine carboxylic acid, piperacillin acid, octahydroindole-2-carboxylic acid, azaprole, 2-amino isobutyric acid or valine, and X is -COOR$^4$, -CONR$^3$R$^6$, 2-thiazole, 2-thiazoline, 2-benzothiazole, 2-oxazole, 2-oxazoline, 2-benzoazole or 2-benzimidazole. More preferred compounds of formula I are those wherein $R^2$ is -NR$^1$R$^2$ and $R^b$ is $R^3$, $R^1$ is -(1-6C)alkylene-COOH or an ester derivative thereof, $R^3$ is (1-12C)alkyl, -CH$_2$-(3-8C)cycloalkyl, dicyclohexylmethyl, diphenylmethyl, or benzyl optionally substituted with one or two substituents selected from halogen, hydroxy or (1-6C)alkoxy; B is lysine; and X is -COOR$^4$, -CONR$^3$R$^6$, 2-thiazole or 2-oxazole. Most preferably A in formula I is proline, 3,4-dehydroproline or azetidine carboxylic acid. Very preferred are compounds of formula I, wherein $R_2$ is $H$; and X is -COOH, and in particular those wherein $R^2$ is -NR$^1$R$^2$ and
R^8 is R^3, R^1 is -CH_2COOH or -CH_2CH_2COOH; R^1 is -CH_2-cyclohexyl, -CH_2-cyclo-octyl, -CH_2-(para-(1-6C)alkoxy-substituted)phenyl or -(CH_2)_5CH_3; A is proline, 3,4-dehydroproline or azetidine carboxylic acid; and B is lysine. Particularly preferred are the compounds HOOC-CH_2-D-cyclohexylalaninyl-prolinyl-lysiny1-COOH and HOOC-CH_2-D-(p-methoxy-phenyl-alanyl)-prolinyl-lysiny1-COOH.

The term (1-6C)alkylene means a branched or unbranched alkylene group having 1 to 6 carbon atoms, such as -(CH_2)_m- and m is 1 to 6, -CH(CH_3)_, -CH(CH_3)-(CH_2)_, etc. Preferred alkylene groups are methylene and ethylene. In ring structures, unbranched (3-6C)alkylene are preferred alkylene groups.

The term (1-12C)alkyl means a branched or unbranched alkyl group having 1 to 12 carbon atoms, such as methyl, ethyl, t-butyl, isopentyl, heptyl, dodecyl, and the like. Preferred alkyl groups are (1-6C)alkyl groups, having 1-6 carbon atoms. Most preferred are (1-4C)alkyl groups, having 1-4 carbon atoms, such as methyl, ethyl, isopropyl, n-butyl and t-butyl.

A (2-12C)alkenyl group is a branched or unbranched unsaturated hydrocarbon group having 2 to 12 carbon atoms. Preferred are (2-6C)alkenyl groups. Examples are ethenyl, propenyl, and the like.

The term (3-8C)cycloalkyl means a cycloalkyl group having 3-8 carbon atoms, being cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl or cyclo-octyl. Cyclopentyl, cyclohexyl and cyclo-octyl are preferred cycloalkyl groups.

A (6-14C)aryl group is an aromatic moiety of 6 to 14 carbon atoms. The aryl group may further contain one or more hetero atoms, such as N, S, or O. Examples of aryl groups are phenyl, naphthyl, (iso)quinolyl, indanyl, and the like. Most preferred is the phenyl group.

A (7-15C)aralkyl group is an alkyl group, substituted by one or more aryl groups, the total number of carbon atoms being 7 to 15, respectively.

The term (1-4C)alkoxy means an alkoxy group having 1-4 carbon atoms, the alkyl moiety of which having the meaning as previously defined.

The term halogen means fluorine, chlorine, bromine or iodine. The preferred halogen is chlorine.

The term ester derivative means any appropriate ester derivative, preferably benzyl- or (1-4C)alkyl-esters such as methyl-, ethyl- or t-butyl-esters.
The term hydrophobic moiety means a (3-8C)cycloalkyl group, a (6-14C)aryl group, or a (1-12C)alkyl group optionally substituted with one or more (3-8C)cycloalkyl groups or (6-14C)aryl groups (which may contain a heteroatom, e.g. nitrogen) such as cyclohexyl, cyclooctyl, phenyl, pyridinyl, naphthyl, tetrahydronaphthyl, and the like. The hydrophobic moiety may further optionally be substituted with substituents such as hydroxy, halogen, trifluoromethyl, (1-4C)alkyl (for instance methyl or ethyl), (1-4C)alkoxy (for instance methoxy), phenyloxy, benzylxy, -OSO₂CF₃, -NH₂CF₃, -NH₂, -NH(1-4C)alkyl, and the like. Preferred hydrophobic moieties are cyclohexyl, cyclohexylmethyl, cyclo-octyl, cyclo-octylmethyl, phenyl, phenylmethyl, naphthyl, naphthylmethyl, p-substituted-phenyl, p-substituted-phenylmethyl (in which both cases the p-substituent is hydroxy, (1-4C)alkoxy or halogen), diphenylmethyl, -(CH₂)₂CH₃, -CH₂CH(CH₃)₂ and -(CH₂)₃CH₃. When R¹ is -NR¹R², the hydrophobic moiety most preferably is cyclohexylmethyl, [p-(1-6C)alkoxy-substituted]phenylmethyl (wherein methoxy is the most preferred substituent) or -(CH₂)₃CH₃.

The invention further includes a process for preparing a compound of formula I, including coupling of suitably protected amino acids or amino acid analogs, followed by removing the protective groups.

The compounds according to formula I may be prepared in a manner conventional for such compounds. To that end, suitably Nα protected (and side-chain protected if reactive side-chains are present) amino acid derivatives or peptides are activated and coupled to suitably carboxyl protected amino acid or peptide derivatives either in solution or on a solid support. Protection of the α-amino functions generally takes place by urethane functions such as the acid-labile tert-butyloxycarbonyl group (Boc), benzylxycarbonyl (Cbz) group and substituted analogs or the base-labile 9-fluorenyl-methylxycarbonyl (Fmoc) group. The Cbz group can also be removed by catalytic hydrogenation. Other suitable amino protective groups include Nps, Bmv, Bpoc, Msc, etc. A good overview of amino protective groups is given is given in The Peptides, Analysis, Synthesis, Biology, Vol. 3 E. Gross and J. Meienhofer, Eds., (Academic Press, New York, 1981). Protection of carboxyl groups can take place by ester formation e.g. base-labile esters like methyl- or ethylesters, acid labile esters like tert-butyesters, or hydrogenolytically-labile esters like benzylesters. Protection of the side chain function of lysine or 4-aminocyclohexylglycine may be accomplished by using the aforementioned groups. Activation
of the carboxyl group of the suitably protected amino acids or peptides can take place by the azide, mixed anhydride, active ester, or carbodiimide method, especially with the addition of catalytic and racemization-suppressing compounds like 1-hydroxybenzotriazole, N-hydroxy-succinimide, 3-hydroxy-4-oxo-3,4-dihydro-1,2,3-benzotriazine, N-hydroxy-5-norbornene-2,3-dicarboximide. See, e.g. The Peptides, Analysis, Synthesis, Biology (see above) and Pure and Applied Chem. 59(3), 331-344 (1987).

The compounds of the invention, which can be in the form of a free base, may be isolated from the reaction mixture in the form of a pharmaceutically acceptable salt. The pharmaceutically acceptable salts may also be obtained by treating the free base of formula I with an organic or inorganic acid such as hydrogen chloride, hydrogen bromide, hydrogen iodide, sulfuric acid, phosphoric acid, acetic acid, propionic acid, glycolic acid, maleic acid, malonic acid, methanesulfonic acid, fumaric acid, succinic acid, tartaric acid, citric acid, benzoic acid, and ascorbic acid.

The compounds of this invention possess one or more chiral carbon atoms, and may therefore be obtained as a pure enantiomer, or as a mixture of enantiomers, or as a mixture containing diastereomers. Methods for obtaining the pure enantiomers are well known in the art, e.g. crystallization of salts which are obtained from optically active acids and the racemic mixture, or chromatography using chiral columns. For diastereomers straight phase or reversed phase columns may be used.

The compounds of the invention may be administered enterally or parenterally, and for humans preferably in a daily dosage of 0.001-100 mg per kg body weight, preferably 0.01-10 mg per kg body weight. Mixed with pharmaceutically suitable auxiliaries, e.g. as described in the standard reference, Gennaro et al., Remington's Pharmaceutical Sciences, (18th ed., Mack Publishing Company, 1990, see especially Part 8: Pharmaceutical Preparations and Their Manufacture) the compounds may be compressed into solid dosage units, such as pills, tablets, or be processed into capsules or suppositories. By means of pharmaceutically suitable liquids the compounds can also be applied in the form of a solution, suspension, emulsion, e.g. for use as an injection preparation, or as a spray, e.g. for use as a nasal spray.
For making dosage units, e.g. tablets, the use of conventional additives such as fillers, colorants, polymeric binders and the like is contemplated. In general any pharmaceutically acceptable additive which does not interfere with the function of the active compounds can be used. Suitable carriers with which the compositions can be administered include lactose, starch, cellulose derivatives and the like, or mixtures thereof, used in suitable amounts.

The invention is further illustrated by the following examples.

**EXAMPLES**

*General:* in the examples the following definitions are used:

- Et = ethyl
- Bzl = benzyl
- iP = isopropyl = 1-methylethyl
- Boc = tert-butyloxycarbonyl
- Cbz = benzoxycarbonyl
- Fmoc = 9-fluorenlymethylxyloxycarbonyl
- Cha = cyclohexylalanyl
- Coa = cyclo-octylalanyl
- Phe = phenylalanyl
- Dpa = 3,3-diphenylalanyl
- Nal = naphthylen-2-yl-alaniny
- Lys = lysinyl
- Nle = norleuciny
- Leu = leucinyl
- Tyr = tyrosiny
- Chg = cyclohexylglycinyl
- Acg = 4-aminocyclohexyl glyciny
- Pro = prolinyl
- DehydroPro = 3,4-dehydroprolinyl
3,3-Dmp = 3,3-dimethyl prolinyl

cisEthylPro = 4-cis-ethyl prolinyl

AzaPro = azaprolinyl

Thz = thiazolidine-4-carboxy

Azt = 2-azetidine carboxy

Pec = pipercolinyl

Ohi = octahydroindole-2-carboxy

Aca = 2-aminocaprylyl

Aib = 2-aminoisobutyryl

Aad = 2-aminoadipyl

EXAMPLE 1

HOOC-CH$_2$-D-Cha-Pro-LysΨ[COCl]$_2$-OH

(a). H-D-Cha-OMe.HCl

To cold (-20 °C) and dry methanol (195 ml) was added dropwise thionylchloride (28 ml). H-D-Cha-OH.HCl (40 g) was added and the reaction mixture was heated under reflux for 5 h. The mixture was concentrated in vacuo and coevaporated with methanol (3 times). The residue was crystallized from methanol/diethylether yielding H-D-Cha-OMe.HCl as a crystalline powder (40.9 g, 95.8%).

TLC: $R_f = 0.66$, silica gel, n-butanol/acetic acid/water=10/1/3 v/v/v.

(b). N-(t-Butyloxy carbonylmethyl)-D-Cha-OMe

t-Butyl-bromo acetate (36 g) was added to a stirred solution of H-D-Cha-OMe.HCl (40.9 g) in 400 ml of acetonitrile. The pH of the mixture was adjusted to 8.5 with diisopropylethylamine. The mixture was stirred for 16 hours at room temperature and evaporated in vacuo. The residue was dissolved in dichloromethane and the solution was washed with water, dried on
sodium sulfate and evaporated in vacuo. Chromatography over silica gel in heptane/ethyl acetate 9/1 (v/v) gave 64 g of N-(t-butyloxycarbonylmethyl)-D-Cha-OMe.
TLC: R<sub>t</sub> = 0.25, silica gel, ethyl acetate/heptane=1/1 v/v.

(c). N-(t-Butyloxycarbonylmethyl)-N-Boc-D-Cha-OMe

The pH of a solution of N-(t-butyloxycarbonylmethyl)-D-Cha-OMe (64 g) and di-t-butyl dicarbonate (40.3 g) in 500 ml N,N-dimethylformamide was adjusted to 8.5 with diisopropylethylamine. The mixture was stirred for 16 hours at room temperature. The solvent was removed in vacuo. Dichloromethane and water were added to the residue. The organic layer was separated, washed with cold 1N hydrogen chloride, water, 5% sodium hydrogen carbonate and water. The organic layer was dried on sodium sulfate and the filtrate was evaporated to an amorphous solid of N-(t-butyloxycarbonylmethyl)-N-Boc-D-Cha-OMe with a yield of 59.6 g.
TLC: R<sub>t</sub>=0.50, silica gel, ethyl acetate/heptane=1/1 v/v.

(d). N-(t-Butyloxycarbonylmethyl)-N-Boc-D-Cha-OH

A solution of N-(t-butyloxycarbonylmethyl)-N-Boc-D-Cha-OMe (59.6 g) in 900 ml of dioxane/water = 9/1 (v/v) was treated with sufficient 6N sodium hydroxide to keep the pH at 12 for 6 hours at room temperature. After acidification, the mixture was poured into water and was extracted with dichloromethane. The organic layer was washed with water and was dried on sodium sulfate. The filtrate was evaporated and yielded 54 g of N-(t-butyloxycarbonylmethyl)-N-Boc-D-Cha-OH.
TLC: R<sub>t</sub>=0.60, silica gel, dichloromethane/methanol = 9/1 v/v

(e). N-(t-Butyloxycarbonylmethyl)-N-Boc-D-Cha-Pro-OBz

To a cold (0 °C) solution of N-(t-butyloxycarbonylmethyl)-N-Boc-D-Cha-OH (13.5 g) in N,N-dimethylformamide (150 ml) were successively added 1-hydroxy benzotriazole (7.09 g), dicyclohexyl carbodiimide (7.61 g), H-Pro-OBzl.HCl (9.31 g) and triethylamine (6 ml). The
mixture was stirred at 0 °C for 1 hour and then kept at room temperature overnight. The mixture was cooled to -20 °C and dicyclohexylurea was removed by filtration. The filtrate was evaporated to dryness. The residue was dissolved in ethyl acetate and washed successively with 5% sodium hydrogen carbonate, water, 3% citric acid and brine, dried over sodium sulfate and concentrated in vacuo. The residue was chromatographed on silica gel in heptane/ethyl acetate = 3/1 (v/v) as eluent. The fractions containing N-(t-Butoxy carbonylmethyl)-N-Boc-D-Cha-Pro-OBzl were pooled and evaporated. Yield: 15 g. TLC: R_f = 0.70, silica gel, heptane/ethyl acetate = 1/1 v/v.

(f). N-(t-Butoxy carbonylmethyl)-N-Boc-D-Cha-Pro-OH

10% palladium on charcoal (750 mg) was added to a solution of N-(t-Butoxy carbonylmethyl)-N-Boc-D-Cha-Pro-OBzl (15 g) in methanol (150 ml). The mixture was hydrogenated at atmospheric pressure at room temperature for 1 hour. The palladium catalyst was removed by filtration and the solvent was removed by evaporation at reduced pressure yielding 11.2 g N-(t-Butoxy carbonylmethyl)-N-Boc-D-Cha-Pro-OH. TLC: R_f = 0.65, silica gel, ethyl acetate/pyridine/acetic acid/water = 213/20/6/11 v/v/v/v.

(g). Cbz-Lys(Boc)-OMe

Cbz-Lys(Boc)-OH (28 g) was dissolved in dichloromethane/methanol = 9/1 v/v (500 ml). 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (23.6 g) was added and the pH of the solution was adjusted to 8 by addition of triethylamine. The reaction mixture was stirred for 2 h at room temperature. The mixture was washed successively with cold 1N hydrogen chloride solution, water, 5% sodium hydrogen carbonate, and water and dried over sodium sulfate. The filtrate was evaporated and the residue was chromatographed on silica gel in ethylacetate/heptane = 1/4 v/v as eluent. The fractions containing Cbz-Lys(Boc)-OMe were pooled and evaporated. Yield: 29.1 g. TLC: R_f = 0.85, silica gel, ethyl acetate/heptane=3/1 v/v.
(h) Cbz-Lys(Boc)\[\text{cyanoacetate}\]

To a cold (-78 °C) solution of Cbz-Lys(Boc)-OMe (29.1 g) in dry dichloromethane (800 ml) was added dropwise diisobutyl aluminium hydride (222 ml of 1M solution in hexane) at a rate to keep the reaction temperature below -70 °C. The resulting solution was stirred at -78 °C for 1 h. A 5% citric-acid solution (600 ml) was added to the reaction mixture. The two layer mixture was stirred at room temperature for 10 minutes, the layers were separated and the aqueous layer was extracted twice with dichloromethane. The combined dichloromethane layers were washed with water and dried over sodium sulfate and filtered. The solution was placed under nitrogen and cooled on an iced-water-bath. A solution of sodium cyanide (36.3 g) and benzyltriethyl ammonium chloride (4.2 g) in water (600 ml) was added. Under vigorous stirring acetic anhydride was added portionwise (2 x 9 ml) over a period of 30 min. The organic layer was separated and the aqueous layer was extracted twice with dichloromethane. The combined dichloromethane layers were washed with water, dried over sodium sulfate, filtered and evaporated in vacuo. The residue was purified by chromatography on silica (eluent: heptane/ethyl acetate = 1/1 v/v) to yield Cbz-Lys (Boc)\[\text{cyanoacetate}\] (26.3 g).

TLC: \( R_f = 0.60 \), silica gel, dichloromethane/ethyl acetate = 7/3 v/v.

(i) Cbz-Lys(Boc)\[\text{CHOHCO}\] - OMe

A solution of Cbz-Lys(Boc)\[\text{cyanoacetate}\] (26.3 g) in diethylether/methanol = 3/1 v/v (600 ml) was cooled to -20 °C under nitrogen, and 66 g of gaseous hydrochloric acid was introduced keeping the temperature below -5 °C. The reaction mixture was kept at 4 °C overnight. Water (100 ml) was added dropwise to the reaction mixture keeping the temperature below 5 °C. After stirring for 16 h at room temperature the organic layer was separated and washed with water. The aqueous layer was saturated with sodium chloride and extracted with sec-butanol/dichloromethane = 3/2 v/v. The organic phase was washed with brine, dried over sodium sulfate, filtered and evaporated in vacuo to give 25.4 g of the crude amine. The residue was taken up in N,N-dimethyl formamide (400 ml), bis-(tert-butyl)anhydride (16 g) was added and triethylamine until pH 8. The reaction mixture was stirred at room temperature overnight. The solvent was removed by evaporation at reduced pressure. The residue was dissolved in
ethyl acetate, washed with water and brine successively, dried over sodium sulfate, filtered and evaporated in vacuo. The residue was purified by chromatography on silica (eluent: ethylacetate/heptane = 4/6 v/v) to yield Cbz-Lys(Boc)Ψ[CHOHCO]-OMe (15.8 g).
TLC: Rf = 0.75, silica gel, ethyl acetate/pyridine/acetic acid/water=63/20/6/11 v/v/v/v.

(j). H-Lys(Boc)Ψ[CHOHCO]-OMe

10% palladium on charcoal (92 mg) and 2 18 ml of a 1N hydrochloride solution were added to a solution of Cbz-Lys(Boc)Ψ[CHOHCO]-OMe (0.92 g) in N,N-dimethyl formamide (20 ml). The mixture was hydrogenated at atmospheric pressure at room temperature for 3 h. The palladium catalyst was removed by filtration and the solvent was removed by evaporation at reduced pressure yielding H-Lys(Boc)Ψ[CHOHCO]-OMe.HCl quantitatively.
TLC: Rf=0.47, silica gel, ethyl acetate/pyridine/acetic acid/water=88/31/18/7 v/v/v/v.

(k). N-(t-Butyloxy carbonylmethyl)-N-Boc-D-Cha-Pro-Lys(Boc)Ψ[CHOHCO]-OMe

To a cold (0 °C) solution of N-(t-Butyloxy carbonylmethyl)-N-Boc-D-Cha-Pro-OH (1.0 g) in N,N-dimethyl formamide (25 ml) were successively added 1-hydroxy benzotriazole (0.42 g), dicyclohexyl carbodiimide (0.47 g) and, after 30 min, H-Lys(Boc)Ψ [CHOHCO]-OMe.HCl (0.9 g) and triethylamine (0.3 ml). The mixture was stirred at 0 °C for 1 hour and then kept at room temperature overnight. The mixture was cooled to -20 °C and dicyclohexylurea was removed by filtration. The filtrate was evaporated to dryness. The residue was dissolved in ethyl acetate and washed successively with 1N hydrogen chloride, water, 5% sodium hydrogen carbonate, water, dried over sodium sulfate and concentrated in vacuo. The residue was chromatographed on silica gel in heptane/ethyl acetate=3/7 (v/v) as eluent. The fractions containing N-(t-Butyloxy carbonylmethyl)-N-Boc-D-Cha-Pro-Lys(Boc)Ψ[CHOHCO]-OMe were pooled and the solvents were evaporated. Yield: 1.16 g
TLC: Rf= 0.28, silica gel, heptane/ethyl acetate= 3/7 v/v.
(l). N-(t-Butyloxy carbonylmethyl)-N-Boc-D-Cha-Pro-Lys(Boc)Ψ[CHOHCO]-OH

N-(t-Butyloxy carbonylmethyl)-N-Boc-D-Cha-Pro-Lys(Boc)Ψ[CHOHCO]-OMe (1.16 g) was dissolved in dioxane/water=7/3 v/v (20 ml) and treated with 0.5M sodium hydroxide solution (3 ml) portionwise over 4 h. at room temperature, keeping the pH at 10-10.5. The reaction mixture was diluted with water (20 ml), 2M hydrogen chloride solution was added until pH 2.0 and the water layer was extracted with dichloromethane. The combined organic phases were washed with water, brine and dried over sodium sulfate, filtered and concentrated in vacuo to yield N-(t-Butyloxy carbonylmethyl)-N-Boc-D-Cha-Pro-Lys(Boc)Ψ[CHOHCO]-OH (1.21 g). TLC: Rf=0.05, silica gel, ethyl acetate/heptane = 8/2 v/v.

(m). N-(t-Butyloxy carbonylmethyl)-N-Boc-D-Cha-Pro-Lys(Boc)Ψ[COCO]-OH

To a solution of N-(t-Butyloxy carbonylmethyl)-N-Boc-D-Cha-Pro-Lys(Boc)Ψ[CHOHCO]-OH (1.21 g) in dry dichloromethane (50 ml) was added 686 mg of periodinane (Dess-Martin reagent). After 1 h stirring at room temperature, 2% sodium thiosulfate solution was added (50 ml) and the mixture was stirred for 30 min at room temperature. The organic layer was separated, washed with water, dried over sodium sulfate, filtered and evaporated in vacuo to give crude N-(t-Butyloxy carbonylmethyl)-N-Boc-D-Cha-Pro-Lys(Boc)Ψ[COCO]-OH (1.38 g). TLC: Rf=0.16, silica gel, ethyl acetate/pyridine/acetic acid/water=163/20/6/11 v/v/v/v.

(n) HOOC-CH₂-D-Cha-Pro-LysΨ[COCO]-OH

N-(t-Butyloxy carbonylmethyl)-N-Boc-D-Cha-Pro-Lys(Boc)Ψ[COCO]-OH (1.38 g, crude) was treated with 60% trifluoroacetic acid/dichloromethane (20 ml) for 4 h at room temperature. The reaction mixture was concentrated in vacuo and the residue dissolved in water and directly charged onto a preparative HPLC DeltaPak RP-C₁₈ using a gradient elution system of 20% A/80% B to 20% A/50% B/30% C over 45 min at a flow rate of 80 ml/min (A: 0.5M phosphate buffer pH 2.1, B: water, C: acetonitril/water=6/4). Yield: 555 mg of HOOC-CH₂-D-Cha-Pro-LysΨ[COCO]-OH

Rt(LC): 23.11 min, A 20 %, B 80 %, C 0 % to A 20 %, B 20 %, C 60 % in 40 min.
EXAMPLE 2

HOOC-CH₂-D-Phe-Pro-Lvsψ(CO-CO)-OH

(a) H-D-Phe-OMe·HCl

To cold (-20 °C) and dry methanol (1 l) was added dropwise thionylchloride (130 ml). H-D-Phe-OH·HCl (147.6 g) was added and the reaction mixture was heated under reflux for 30 min and then kept at room temperature overnight. The mixture was concentrated in vacuo and coevaporated with methanol (3 times). The residue was crystallized from methanol/diethylether yielding H-D-Phe-OMe·HCl as a crystalline powder (187.4 g).
TLC: Rₜ = 0.54, silica gel, n-butanol/acetic acid/water = 10/1/3 v/v/v.

(b) N-(t-Butyloxy carbonylmethyl)-D-Phe-OMe

t-Butyl-bromo acetate (65 ml) was added to a stirred solution of H-D-Phe-OMe·HCl (65.2 g) in 400 ml of acetonitrile. The pH of the mixture was adjusted to 8.5 with diisopropylethylamine. The mixture was stirred for 16 hours at room temperature and evaporated in vacuo. The residue was dissolved in dichloromethane and the solution was washed with water, dried on sodium sulfate and evaporated in vacuo. Chromatography over silica gel in heptane/ethyl acetate 9/1 (v/v) gave 96.4 g of N-(t-butyloxy carbonylmethyl)-D-Phe-OMe.
TLC: Rₜ = 0.90, silica gel, ethylacetate/pyridine/acetic acid/water = 376/31/18/7 v/v/v/v.

(c) N-(t-Butyloxy carbonylmethyl)-N-Boc-D-Phe-OMe

The pH of a solution of N-(t-butyloxy carbonylmethyl)-D-Phe-OMe (96.4 g) and di-t-butyl dicarbonate (72.2 g) in N,N-dimethylformamide (400 ml) was adjusted to 8.5 with diisopropylethylamine. The mixture was stirred for 48 hours at room temperature. The solvent was removed in vacuo. Dichloromethane and water were added to the residue. The organic layer was separated, washed with cold 1N hydrogen chloride, water, saturated sodium hydrogen carbonate solution and water. The organic layer was dried on sodium sulfate and the
filtrate was evaporated. The residue was chromatographed on silica in toluene/ethyl acetate = 9/1 (v/v) as eluent. The fractions containing N-(t-Butyloxy carbonylmethyl)-N-Boc-D-Phe-OMe were pooled and evaporated. Yield: 115.3 g.

TLC: Rf = 0.77, silica gel, toluene/ethyl acetate = 9/1 v/v.

(d). N-(t-Butyloxy carbonylmethyl)-N-Boc-D-Phe-OH

A solution of N-(t-Butyloxy carbonylmethyl)-N-Boc-D-Phe-OMe (115.3 g) in 800 ml of dioxane/water = 9/1 (v/v) was treated with sufficient 2N sodium hydroxide to keep the pH at 12 for 16 hours at room temperature. After acidification, the mixture was poured into water and was extracted with dichloromethane. The organic layer was washed with water and was dried on sodium sulfate. The filtrate was evaporated and yielded 104 g of N-(t-Butyloxy carbonylmethyl)-N-Boc-D-Phe-OH.

TLC: Rf = 0.10, silica gel, toluene/ethyl acetate = 7/3 v/v.

(e). N-(t-Butyloxy carbonylmethyl)-N-Boc-D-Phe-Pro-OBzl

To a cold (0 °C) solution of N-(t-Butyloxy carbonylmethyl)-N-Boc-D-Phe-OH (5.3 g) in N,N-dimethyl formamide (40 ml) were successively added 1-hydroxy benzotriazole (2.8 g), dicyclohexyl carbodiimide (3.2 g), H-Pro-OBzl.HCl (3.78 g) and triethylamine (2.16 ml). The mixture was stirred at 0 °C for 1 hour and then kept at room temperature overnight. The mixture was cooled to -20 °C and dicyclohexylurea was removed by filtration. The filtrate was evaporated to dryness. The residue was dissolved in ethyl acetate and washed successively with 5% sodium hydrogen carbonate, water, 2% citric acid and brine, dried over sodium sulfate and concentrated in vacuo. The residue was chromatographed on silica gel in heptane/ethyl acetate = 6/4 (v/v) as eluent. The fractions containing N-(t-Butyloxy carbonylmethyl)-N-Boc-D-Phe-Pro-OBzl were pooled and evaporated. Yield 4.35 g.

TLC: Rf = 0.74, silica gel, heptane/ethyl acetate = 1/1 v/v.
(f). N-(t-Butyloxy carbonylmethyl)-N-Boc-D-Phe-Pro-OH

10% palladium on charcoal (450 mg) was added to a solution of N-(t-Butyloxy carbonylmethyl)-N-Boc-D-Phe-Pro-OBzI (4.35 g) in methanol (50 ml). The mixture was hydrogenated at atmospheric pressure at room temperature for 45 min. The palladium catalyst was removed by filtration and the solvent was removed by evaporation at reduced pressure yielding 3.48 g of N-(t-Butyloxy carbonylmethyl)-N-Boc-D-Phe-OH.
TLC: Rf = 0.63, silica gel, ethyl acetate/pyridine/acetic acid/water = 664/31/18/7 v/v/v/v.

(g). N-(t-Butyloxy carbonylmethyl)-N-Boc-D-Phe-Pro-Lys(Boc)Ψ[CHOHCO]-OMe

The compound was prepared according to example 1(k). Yield: 388 mg.
TLC: Rf = 0.69, silica gel, dichloromethane/methanol = 9/1 v/v.

(h). N-(t-Butyloxy carbonylmethyl)-N-Boc-D-Phe-Pro-Lys(Boc)Ψ[CHOHCO]-OH

The compound was prepared according to example 1(l). Yield: 260 mg.
TLC: Rf = 0.14, silica gel, dichloromethane/methanol = 9/1 v/v.

(i). N-(t-Butyloxy carbonylmethyl)-N-Boc-D-Phe-Pro-Lys(Boc)Ψ[COCO]-OH

The compound was prepared according to example 1(m). Yield: 259 mg.
TLC: Rf = 0.10, silica gel, ethyl acetate/pyridine/acetic acid/water = 188/31/18/7 v/v/v/v.

(j). HOOC-CH₂-D-Phe-Pro-LysΨ[COCO]-OH

N-(t-Butyloxy carbonylmethyl)-N-Boc-D-Phe-Pro-Lys(Boc)Ψ[COCO]-OH (259 mg, crude) was treated with trifluoroacetic acid (10 ml) for 2 h at room temperature. The reaction mixture was concentrated in vacuo and the residue dissolved in water and directly charged onto a preparative HPLC DeltaPak RP-C₁₈ using a gradient elution system of 20 % A / 80 % B to 20 % A / 0 % B / 80 % C over 40 min at a flow rate of 80 ml/min (A: 0.5M phosphate buffer
pH 2.1, B: water, C: acetonitril/water = 6/4). Yield: 61 mg of HOOC-CH_2-D-Phe-Pro-Lys[COCO]-OH.
Rt(LC): 17.11 min, A 20 %, B 80 %, C 0 % to A 20 %, B 20 %, C 60 % in 40 min.

**EXAMPLE 3**

**HOOC-CH_2-D-Cha-Pro-Lys-Ψ[COCO]-OEt**

(a).Cbz-Lys(Boc)Ψ[CHOHCO]-OEt

A solution of Cbz-Lys(Boc)Ψ(cyanoacetate) (21.9 g) in diethyl ether/ethanol = 3/1 v/v (600 ml) was cooled to -20 °C under nitrogen, and 65.6 g of gaseous hydrochloric acid was introduced keeping the temperature below -5 °C. The reaction mixture was kept at 4 °C overnight. Water was added (102 ml) dropwise to the reaction mixture keeping the temperature below 5 °C. After stirring for 4 h at room temperature the organic layer was separated and washed 3 times with water. The pH of the water layer was adjusted to 8.5 with sodium hydrogen carbonate and extracted with sec-butanol/dichloromethane = 1/1 v/v. The organic phase was washed with water and brine and dried over magnesium sulfate, filtered and evaporated in vacuo to give 20 g of the crude amine.

TLC: Rf=0.22 and 0.29 (diastereomers), silica gel, ethyl acetate/pyridine/acetic acid/water = 63/20/6/11 v/v/v/v.

The residue was taken up in N,N-dimethylformamide (300 ml) and the pH was adjusted to 8.5 with triethyl amine followed by addition of bis-(t-butyl)anhydride (13 g). The reaction mixture was stirred at 4 °C during 64 h. The solvent was removed by evaporation in vacuo and the residue was dissolved in ethyl acetate, washed with water and brine, dried over sodium sulfate, filtered and evaporated in vacuo. The residue was purified by chromatography on silica (eluent heptane/ethyl acetate 2/8 v/v) followed by a second purification on silica (eluent toluene/ethyl acetate 6/4 v/v) (4.2 g).

TLC: Rf=0.42 and 0.46 (diastereomers), silica gel, toluene/ethyl acetate 7/3 v/v.
(b). H-Lys(Boc)Ψ[CHOHCO]-OEt

10% palladium on charcoal (381 mg) and 2.53 ml of a 2N hydrochloride solution were added to a solution of Cbz-Lys(Boc)Ψ[CHOHCO]-OEt (2.2 g) in ethanol (80 ml). The mixture was hydrogenated at atmospheric pressure at room temperature for 45 minutes. The palladium catalyst was removed by filtration and the solvent was removed by evaporation at reduced pressure yielding H-Lys(Boc)Ψ[CHOHCO]-OEt HCl quantitatively. TLC: Rf=0.10, silica gel, dichloro methane/methanol 9/1 v/v.

(c). N-(t-Butyloxy carbonylmethyl)-N-Boc-D-Cha-Pro-Lys(Boc)Ψ[CHOHCO]-OEt

To a cold (0 °C) solution of N-(t-Butyloxy carbonylmethyl)-N-Boc-D-Cha-Pro-OH (2.46 g) in N,N-dimethyl formamide (20 ml) were successively added 1-hydroxy benzotriazole (0.76 g), dicyclohexyl carbodiimide (1.16 g) and, after preactivating this mixture for one hour, H-Lys(Boc)Ψ[CHOHCO]-OEt HCl (1.72 g) dissolved in 10 ml dimethyl formamide, pH adjusted to 8-9 with diisopropyl ethylamine, was added. The mixture was stirred at 0 °C for 1 hour and then kept at room temperature for three hours. After that the mixture was cooled to 0 °C and additional 0.2 eq of dicyclohexyl carbodiimide and 1-hydroxy benzotriazole were added. After 2 hours at room temperature the reaction appeared completed. The mixture was cooled to -20 °C and dicyclohexylurea was removed by filtration. The filtrate was evaporated to dryness. The residue was dissolved in ethyl acetate and washed successively with 5 % sodium hydrogen carbonate, water, 1N hydrogen chloride, and water. Then the residue was dried over sodium sulfate and concentrated in vacuo. The residue was chromatographed on silica gel in dichloro methane/ethanol = 95/5 (v/v) as eluent. The fractions containing N-(t-butyloxy carbonylmethyl)-N-Boc-D-Cha-Pro-Lys(Boc)Ψ[CHOHCO]-OEt were pooled and evaporated to dryness. Yield: 3.30 g.
TLC: Rf= 0.85, silica gel, dichloromethane/methanol = 9/1 v/v.

(d). N-(t-Butyloxy carbonylmethyl)-N-Boc-D-Cha-Pro-Lys(Boc)Ψ[COCO]-OEt

The compound was prepared according to example 1. Yield: 395 mg.
TLC: Rf = 0.12, silica gel, dichloro methane/methanol = 97/3 v/v.

(e) HOOC-CH₂-D-Cha-Pro-LysΨ[COCO]-OEt

N-(t-Butyloxy carbonylmethyl)-N-Boc-D-Cha-Pro-Lys(Boc)Ψ[COCO]-OEt (395 mg, crude) was treated with 57% trifluoroacetic acid/dichloromethane (7 ml) for 4 h at room temperature. The reaction mixture was concentrated in vacuo and the residue dissolved in water, filtered and lyophilized. Yield 370 mg of the end product as ditrifluoro acetate salt. TLC: Rf = 0.49, silica gel, ethyl acetate/pyridine/acetic acid/water = 44/31/18/7 v/v/v/v. HPLC purification, as described in example 1, afforded the pure end product in 130 mg yield. Rt(LC): 32.17 min, A 20 %, B 80 %, C 0 % to A 20 %, B 20 %, C 60 % in 40 min.

EXAMPLE 4

(HOOC-CH₂)₂-D-Cha-Pro-Lys-(2-thiazolyl)

(a) Boc-D-Cha-Pro-OBzl

The compound was prepared according to example 1. Yield: 34.24 g.

TLC: Rf = 0.18, silica gel, heptane/ethyl acetate = 7/3 v/v.

(b) Boc-D-Cha-Pro-OH

The compound was prepared according to example 1 with a reaction time of five hours. Yield: 26.61 g.

TLC: Rf = 0.1, silica gel, heptane/ethyl acetate = 1/1 v/v.

(c) Boc-Lys(Cbz)-NMeOMe

Boc-Lys(Cbz)-OH, dicyclohexylamine (10 g, 17.8 mmol) was suspended in dichloromethane (200 ml). The suspension was washed with cold 0.1N hydrogen chloride solution twice.
2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (6.0 g, 18.7 mmol) and 
O,N-dimethyl-hydroxylamine.HCl (1.82 g, 18.7 mmol) were added to the resulting organic 
phase and the pH was adjusted to 8 by adding triethylamine. The reaction mixture was stirred 
for 1 h at room temperature. The mixture was washed successively with cold 2N hydrochloric 
acid solution, water, 5 % sodium hydrogen carbonate solution, and water. The organic layer 
was dried over sodium sulfate, filtered and evaporated. The residue was purified by 
chromatography on silica (eluent: dichloromethane/methanol = 95/5 v/v) to yield 7.2 g.
TLC : $R_f = 0.55$, silica gel, dichloromethane/methanol = 95/5 v/v

(d) Boc-Lys(Cbz)-(2-thiazolyl)

To a cold (-78 °C), stirred solution of n-buthyl lithium (63.9 mmol) in diethyl ether (58 ml), was 
added, dropwise, a solution of 2-bromothiazole (10.5 g, 63.9 mmol) in diethyl ether (30 ml). 
After the solution had been stirred at -78 °C for 30 min, a solution of Boc-Lys(Cbz)-NMeOMe 
(8.2 g, 19.4 mmol) in dry tetrahydrofuran (THF) (75 ml) was added slowly. The mixture was 
stirred at -78 °C for 1 h, then 5% aqueous sodium hydrogen carbonate was added. The mixture 
was allowed to warm to room temperature and the layers were separated. The aqueous layer 
was extracted with diethyl ether. The combined organic layers were washed with water, dried 
over sodium sulfate, filtered and evaporated. The residue was purified by chromatography on 
silica (eluent: ethyl acetate/heptane = 3/1 v/v) to yield 8.6 g.
TLC: $R_f = 0.77$, silica gel, ethyl acetate/heptane = 3/1 v/v

(e) H-Lys(Cbz)-(2-thiazolyl) TFA

Boc-Lys(Cbz)-(2-thiazolyl) (2.78 g) was dissolved in 50% trifluoroacetic acid (TFA) 
dichloromethane (50 ml) and stirred for 1 hour at room temperature. The crude amine was 
isolated as a yellow oil in quantitative yield after removal of the solvent by evaporation, and 
used immediately to prepare Boc-D-Cha-Pro-Lys(Cbz)-(2-thiazolyl).
TLC : $R_f = 0.25$, silica gel, ethyl acetate/pyridine/acetic acid/water = 63/20/6/11 v/v/v/v.
(f). Boc-D-Cha-Pro-Lys(Cbz)-(2-thiazolyl)

Boc-D-Cha-Pro-OH (2.3 g) was dissolved in dry dimethyl formamide (75 ml). After addition of ethyl diisopropyl amine (2.16 ml), the reaction mixture was placed under nitrogen and cooled to -15 °C. Isobutyl chloroformate (806 µl) was subsequently added and the mixture was allowed to stir for 15 min at -15 °C. H-Lys(Cbz)-(2-thiazolyl) TFA was dissolved in dry dimethylformamide (40 ml) and added dropwise to the cold mixed anhydride solution, maintaining the pH at 8.5 by addition of ethyl diisopropyl amine. The reaction mixture was stirred for 30 min at -15 °C. The reaction mixture was evaporated to dryness. The residue was dissolved in ethylacetate and successively washed with water, 5% aqueous sodium hydrogen carbonate solution, water, brine, and was dried over sodium sulfate and concentrated in vacuo. The residue was purified by chromatography on silica (eluent: heptane/ethyl acetate = 4/6 v/v) to yield Boc-D-Cha-Pro-Lys(Cbz)-(2-thiazolyl) (3.24 g).

TLC: Rf = 0.50, silica gel, dichloromethane/methanol = 9/1 v/v.

(g). H-D-Cha-Pro-Lys(Cbz)-(2-thiazolyl) TFA

Boc-D-Cha-Pro-Lys(Cbz)-(2-thiazolyl) (318 mg) was dissolved in 50% trifluoroacetic acid/dichloromethane (3 ml) and stirred for 1 hour at room temperature. The crude amine was isolated as a yellow oil in quantitative yield after removal of the solvent by evaporation, and used immediately to prepare N-(di-t-Butyloxy carbonylmethyl)-D-Cha-Pro-Lys(Cbz)-(2-thiazolyl).

(h). N-(di-t-Butyloxy carbonylmethyl)-D-Cha-Pro-Lys(Cbz)-(2-thiazolyl)

t-Butyl-bromo acetate (0.68 ml) was added to a stirred solution H-D-Cha-Pro-Lys(Cbz)-(2-thiazolyl) TFA in 5 ml of acetonitrile. The pH of the mixture was adjusted to 8 with diisopropylethylamine. The mixture was stirred for 3.5 hours at room temperature, refluxed for 6 hours at 45 °C and evaporated in vacuo. The residue was dissolved in ethyl acetate and the solution was washed with water, dried on sodium sulfate and evaporated in vacuo.
Chromatography over silica gel in toluene/ethyl acetate 1/2 (v/v) gave 287 mg of N-(di-t-butyloxycarbonylmethyl)-D-Cha-Pro-Lys(Cbz)-(2-thiazolyl).
TLC: \( R_f = 0.50 \), silica gel, toluene/ethyl acetate = 1/1 v/v.

(i) \((\text{HOOC-CH}_2)_2\)-D-Cha-Pro-Lys-(2-thiazolyl)

N-(di-t-Butyloxycarbonylmethyl)-D-Cha-Pro-Lys(Cbz)-(2-thiazolyl) (287 mg) was treated with trifluoroacetic acid/thioanisole = 10/1 (v/v) (3.85 ml) for 4 h at room temperature. The reaction mixture was concentrated in vacuo and the residue dissolved in water. The aqueous phase was washed extensively with diethyl ether. The water layer was directly charged onto a preparative HPLC DeltaPak RP-C\(_{18}\) using a gradient elution system of 20% A/70% B/10% C to 20% A/30% B/50% C over 45 min at a flow rate of 50 ml/min. Yield: 120 mg of \((\text{HOOC-CH}_2)_2\)-D-Cha-Pro-Lys-(2-thiazolyl).
Rt(LC): 34.21 min, A: 20%, B: 80%, C: 0% to A: 20%, B: 20%, C: 60% in 40 min.

EXAMPLE 5

\textbf{HOOC-CH}_2\text{-D-Cha-Ohi-Lys-(2-thiazolyl)}

(a) H-Ohi-OMe HCl

To cold (-15 °C) and dry methanol (10 ml) 0.43 ml (0.7 g) of SOCl\(_2\) was added dropwise. The mixture was stirred for 20 min at -10 °C after which octahydroindolin-2-carboxylic acid (0.5 g) was added and the solution was refluxed for 3 h. The mixture was concentrated and coevaporated with methanol in vacuo and yielded 665 mg H-Ohi-OMe HCl.
TLC: \( R_f = 0.68 \), silicagel, butanol/pyridine/acetic acid/water 4/1/1/2 v/v/v/v.

(b) N-(t-Butyloxycarbonylmethyl)-N-Boc-D-Cha-Ohi-OMe

To a cold (0 °C) solution of N-(t-butyloxycarbonylmethyl)-N-Boc-D-Cha-OH (1.2 g) in dichloro methane (40 ml) were successively added 1-hydroxy benzotriazole (460 mg),
dicyclohexyl carbodiimide (700 mg) and stirred for 20 min at 0 °C. Next H-OhO-OMe.HCl (665 mg) and diisopropyl ethylamine (0.075 ml) were added to this reaction mixture. The mixture was stirred at 0 °C for 1 hour and then kept at room temperature during 72 h keeping the pH of the solution at 7. The mixture was cooled to -20 °C and dicyclohexylurea was removed by filtration. The filtrate was evaporated to dryness. The residue was dissolved in ethyl acetate and washed successively with 1N HCl, saturated sodium hydrogen carbonate, water and brine, dried over sodium sulfate and concentrated in vacuo. The residue was chromatographed on silicagel in heptane/ethyl acetate = 3/1 (v/v) as eluent. The fractions containing N-(t-butyloxy-carbonylmethyl)-N-Boc-D-Cha-Ohi-OMe were pooled and evaporated. Yield: 1.07 g.

TLC: Rf = 0.41, silica gel, heptane/ethyl acetate = 3/1 v/v.

(c) N-(t-Butyloxy carbonylmethyl)-N-Boc-D-Cha-Ohi-OH

To a solution of N-(t-butyloxy-carbonylmethyl)-N-Boc-D-Cha-Ohi-OMe (1.05 g) in dioxane/water 1/1 v/v (40 ml) LiOH.H₂O (0.32 g) was added. The mixture was stirred at room temperature for 6.5 h. Solvent was removed by evaporation at reduced pressure, ethyl acetate was added, the pH was adjusted to 1.5 and the water layer was extracted three times with ethyl acetate. The organic layer was washed with water and brine, dried on sodium sulfate and concentrated in vacuo. The residue was chromatographed on silicagel in heptane/ethyl acetate = 1/2 (v/v) as eluent. The fractions containing N-(t-butyloxy carbonylmethyl)-N-Boc-D-Cha-Ohi-OH were pooled and evaporated. Yield: 500 mg.

TLC: Rf = 0.25, silica gel, ethyl acetate/heptane 2/1 v/v.

(d) N-(t-Butyloxy carbonylmethyl)-N-Boc-D-Cha-Ohi-Lys(Cbz)-(2-thiazolyl)

The compound was prepared according to example 4. Yield: 431 mg.

TLC: Rf = 0.69, silica gel, ethyl acetate/heptane 2/1 v/v.
(e) **HOOC-CH₂-D-Cha-Ohi-Lys-(2-thiazoly).**

N-t-Butyloxy carbonylmethyl-N-Boc-D-Cha-Ohi-Lys(Cbz)-(2-thiazoly) (431 mg) was treated with trifluoroacetic acid/thioanisole = 10/1 (v/v) (25 ml) for 4 h at room temperature. The reaction mixture was concentrated in vacuo and the residue dissolved in water. The aqueous phase was washed extensively with diethyl ether. The water layer was lyophilized.

Yield: 150 mg of crude HOOC-CH₂-D-Cha-Ohi-Lys-(2-thiazoly).

The crude product was charged onto a preparative HPLC DeltaPak RP-C₁₈ using a gradient elution system of 20% A/70% B/10% C to 20% A/30% B/50% C over 45 min at a flow rate of 50 ml. Yield: 124 mg of HOOC-CH₂-D-Cha-Ohi-Lys-(2-thiazoly)

Rt(LC): 41.50 min. A: 20%, B: 80%, C: 0% to A: 20%, B: 20%, C: 60% in 40 min.

**EXAMPLE 6**

**HOOC-CH₂-D-Cha-cisEthylPro-Lys-(2-thiazoly)**

(a) **N-(t-Butyloxy carbonylmethyl)-N-Boc-D-Cha-cisEthylPro-OEt**

To a cold (0 °C) solution of N-(t-butyloxy carbonylmethyl)-N-Boc-D-Cha-OH (3.7 g) in dichloromethane (40 ml) were successively added 1-hydroxy benzotriazole (1.43 g), dicyclohexyl carbodiimide (2.18 g) and stirred for 15 min at 0 °C. Next H-cisEthylPro-OEt·HCl (1.99 g), which was prepared according to WO 95/23608, and diisopropyl ethylamine (1.50 ml) were added to this reaction mixture. The mixture was stirred at 0 °C for 1 hour and then kept at room temperature during 2 h keeping the pH of the solution at 7. The mixture was cooled to -20 °C and dicyclohexylurea was removed by filtration. The filtrate was evaporated to dryness. The residue was dissolved in ethyl acetate and washed successively with 1N hydrogen chloride, saturated sodium hydrogen carbonate, water and brine, dried over sodium sulfate and concentrated in vacuo. The residue was chromatographed on silicagel in heptane/ethyl acetate = 3/1 (v/v) as eluent. The fractions containing N-(t-butyloxy carbonylmethyl)-N-Boc-D-Cha-cisEthylPro-OEt were pooled and evaporated.

Yield: 3.87 g (75%).

TLC: Rₜ= 0.46, silica gel, heptane/ethyl acetate= 3/1 v/v.
(b) \textit{N-}(t-\text{Butyloxy carbonylmethyl})-\textit{N-Boc-D-Cha-cisEthylPro-OH}

To a solution of \textit{N-}(t-\text{Butyloxy carbonylmethyl})-\textit{N-Boc-D-Cha-cisEthylPro-OEt} (3.85 g) in dioxane/water 9/1 v/v (50 ml) 1N sodium hydroxide (7.5 ml) was added. The mixture was stirred at room temperature for 72 h. After 60 h 70 mg LiOH.H2O in 10 ml of water was added. The reaction mixture was diluted with 100 ml of water, the pH was adjusted to 1.5 and the water layer was extracted three times with dichloro methane. The organic layer was washed with water and brine, dried on sodium sulfate and concentrated in vacuo. The residue was chromatographed on silicagel in heptane/ethyl acetate = 1/2 (v/v) as eluent. The fractions containing \textit{N-}(t-\text{Butyloxy carbonylmethyl})-\textit{N-Boc-D-Cha-cisEthylPro-OH} were pooled and evaporated. Yield: 1.39g.

TLC: \textit{R}_f = 0.79, silica gel, ethyl acetate/pyridine/acetic acid/water 126/20/11/6 v/v/v/v.

(e) \textit{N-}(t-\text{Butyloxy carbonylmethyl})-\textit{N-Boc-D-Cha-cisEthylPro-Lys(Cbz)-(2-thiazolyl)}

The compound was prepared according to example 4. Yield: 403 mg.

TLC : \textit{R}_f = 0.66, silica gel, ethyl acetate/heptane 2/1 v/v.

(d) \textit{HOOC-CH}_2-\textit{D-Cha-cisEthylPro-Lys-(2-thiazolyl)}

\textit{N-t-Butyloxy carbonylmethyl-N-Boc-D-Cha-cisEthylPro-Lys(Cbz)-(2-thiazolyl)} (403 mg) was treated with trifluoroacetic acid/thioanisole = 10/1 (v/v) (25 ml) for 4 h. at room temperature. The reaction mixture was concentrated in vacuo and the residue dissolved in water. The aqueous phase was washed extensively with diethylether. The water layer was lyophilized. The crude product was charged onto a preparative HPLC DeltaPak RP-C18 using a gradient elution system of 20\% A/70\% B/10\% C to 20\% A/30\% B/50\% C over 45 min at a flow rate of 50 ml. Yield: 140 mg of \textit{HOOC-CH}_2-D-Cha-cisEthylPro-Lys-(2-thiazolyl).

\textit{Rt}(LC): 39.52 min, A: 20\%, B: 80\%, C: 0\% to A: 20\%, B: 20\%, C: 60\% in 40 min.
EXAMPLE 7

HOOC-CH₂-D-p-OCH₃-Phe-Pro-LysΨ[COCO]-OH

(a) N-(t-Butyloxycarbonylmethyl)-N-Boc-D-p-OCH₃-Phe-Pro-OH

According to the same procedures as described in example 1, H-D-p-OCH₃-Phe-OH (4.97 g) was converted into N-(t-Butyloxycarbonylmethyl)-N-Boc-D-p-OCH₃-Phe-Pro-OH (5.33 g).
TLC: Rf = 0.59, silica gel, ethyl acetate/pyridine/acetic acid/water = 213/20/6/11 v/v/v/v.

(b) N-(t-Butyloxycarbonylmethyl)-N-Boc-D-p-OCH₃-Phe-Pro-Lys(Boc)Ψ[CHOHCO]-OMe

Coupling of N-(t-Butyloxycarbonylmethyl)-N-Boc-D-p-OCH₃-Phe-Pro-OH (443.5 mg) with H-Lys(Boc)Ψ[CHOHCO]-OMe HCl (306.9 mg) was performed as described in example 1. Silica gel purification, using dichloromethane/methanol 99/1 → 95/5 v/v, afforded 685.5 mg N-(t-Butyloxycarbonylmethyl)-N-Boc-D-p-OCH₃-Phe-Pro-Lys(Boc)Ψ[CHOHCO]-OMe.
TLC: Rf = 0.85, silica gel, ethyl acetate/methanol = 9/1 v/v.

(c) HOOC-CH₂-D-p-OCH₃-Phe-Pro-LysΨ[COCO]-OH

According to the procedures as described in examples 1, N-(t-Butyloxycarbonylmethyl)-N-Boc-D-p-OCH₃-Phe-Pro-Lys(Boc)Ψ[CHOHCO]-OMe (685 mg) was converted into HOOC-CH₂-D-p-OCH₃-Phe-Pro-LysΨ[COCO]-OH (638 mg, crude). Preparative HPLC purification on a DeltaPak RP-C₁₈ column, using a gradient elution system of 20% A/80% B to 20% A/50% B/30% C over 45 min at a flow rate of 80 ml/min afforded 340 mg of the target compound.
Rt(LC): 18.89 min, A 20 %, B 80 %, C 0 % to A 20 %, B 20 %, C 60 % in 40 min

EXAMPLE 8

HOOC-CH₂-D-m-Cl-Phe-Pro-LysΨ[COCO]-OH
(a) Diethyl 2-acetylamino-2-(3-chlorophenylmethyl)-malonate

Dry ethanol (100 ml) was added dropwise (very slow) to a cold (0°C) suspension of sodium hydride (4 g, 60 % dispersion in oil) in dioxane (20 ml) and stirred for 1.5 hours at 0°C under a nitrogen atmosphere. This resulting sodium ethanolate solution was added dropwise to a solution of 3-chlorobenzyl chloride (16.91 g), sodium iodide (1.5 g) and diethyl acetylaminomalonate (22.8 g) in dioxane (200 mL)/ethanol (20 mL) and stirred vigorously for 16 hours at 95 °C. The solvent was removed by evaporation at reduced pressure, ethyl acetate was added, washed with water, 5% sodium hydrogencarbonate solution and brine, dried on sodium sulphate and concentrated in vacuo. The product was crystallised from ethyl acetate/hexane, filtered, washed with hexane and dried to afford diethyl 2-acetylamino-2-(3-chlorophenylmethyl)-malonate

Yield: 28.15 g.

TLC: Rf = 0.56 , silica gel, heptane/ethyl acetate 1/1 v/v.

(b) H-D,L-m-Cl-Phe-OH

The amino acid H-D,L-m-Cl-Phe-OH was obtained by refluxing a solution of diethyl 2-acetylamino-2-(3-chlorophenylmethyl)-malonate (28.15 g) in 4N hydrochloric acid/acetic acid 2:1 v/v (240 ml) for 16 hours. The reaction mixture was cooled and the solvent was evaporated to dryness. The crude reaction product was coevaporated with toluene (3x) and with methanol (2x). Yield: 20.15 g.

TLC: Rf = 0.59 , silica gel, ethyl acetate/pyridine/acetic acid/water 63/20/6/11 v/v/v/v.

(c) N-(t-Butoxycarbonylmethyl)-N-Boc-D,L-m-Cl-Phe-Pro-OMe

H-D,L-m-Cl-Phe-OH was converted into N-(t-Butoxycarbonylmethyl)-N-Boc-D,L-m-Cl-Phe-OH according to the procedures described in examples 1 (a - d). Coupling of N-(t-Butoxycarbonylmethyl)-N-Boc-D,L-m-Cl-Phe-OH (12.5 g) with H-Pro-OMe.HCl (5.5 g), under the conditions described in example 1 (e), afforded 14.7 g pure protected dipeptide after silica gel purification (eluent: dichloromethane/methanol 98/2 v/v).
TLC: \( R_f = 0.87 \), silica gel, ethyl acetate/pyridine/acetic acid / water = 213/20/6/11 v/v/v/v.

(d) \( \text{N-\{(t-Butyloxycarbonylmethyl)-N-Boc-D,L-m-Cl-Phe-Pro-OH} \)

A solution of \( \text{N-\{(t-Butyloxycarbonylmethyl)-N-Boc-D,L-m-Cl-Phe-Pro-OMe} (14.7 \text{ g}) \) in 200 ml of dioxane/water = 9/1 (v/v) was treated with sufficient 1N sodium hydroxide to keep the pH at 10 - 10.5, until the starting material had disappeared. The reaction mixture was diluted with water, acidified with aqueous hydrochloric acid (2N) to pH = 2 and extracted with dichloromethane (3x). The organic layers were pooled, washed with water and dried over sodium sulfate. After evaporation of the solvent in vacuo, 14.4 g crude product was isolated.

TLC: \( R_f = 0.43 \), silica gel, ethyl acetate/pyridine/acetic acid / water = 213/20/6/11 v/v/v/v.

(e) \( \text{HOOC-CH}_2\text{-D-m-Cl-Phe-Pro-Lys}\Psi[\text{COCO}]-\text{OH} \)

In analogy to example 1, \( \text{N-\{(t-Butyloxycarbonylmethyl)-N-Boc-D,L-m-Cl-Phe-Pro-OH} (879 mg) \) was coupled with \( \text{H-Lys(Boc)Ψ[CHOHCO]-OMe.HCl} \) (641 mg) to afford the fully protected tripeptide (1.1 g) after silica gel purification. Saponification, followed by Dess-Martin oxidation and subsequent deprotection, as in examples 1 gave 948 mg crude \( \text{HOOC-CH}_2\text{-D-mCl-Phe-Pro-LysΨ[COCO]} \)-OH. A part of this crude product (365 mg) was purified by HPLC on a DeltaPak RP-C18 column, under the conditions described in example 1, to obtain the desired product as a single isomer (113 mg).

Rt(LC): 22.83 min, A 20 %, B 80 %, C 0 % to A 20 %, B 20 %, C 60 % in 40 min.

EXAMPLE 9

\( \text{HOOC-CH}_2\text{-D-Dpa-Pro-LysΨ[COCO]}-\text{OH} \)

(a) \( \text{N-\{(t-Butyloxycarbonylmethyl)-N-Boc-D-Dpa-Pro-OH} \)

H-D-Dpa-OH.HCl (10 g) was converted into \( \text{N-\{(t-Butyloxycarbonylmethyl)-N-Boc-D-Dpa-Pro-OH} (4.3 \text{ g}) \), according to the methods as described in examples 1.
TLC: $R_f = 0.55$, silica gel, ethyl acetate/pyridine/acetic acid/water = 563/20/6/11 v/v/v/v.

(b) **HOOC-CH$_2$-D-Dpa-Pro-LysΨ[COCO]-OH**

N-(t-Butyloxycarbonylmethyl)-N-Boc-D-Dpa-Pro-OH (420 mg) was coupled with H-Lys(Boc)Ψ[CHOHCO]-OMe.HCl (250 mg), under the conditions as described in example 1. Silica gel purification in heptane/ethyl acetate 1/1 → 1/4 (v/v) afforded 482 mg of the fully protected tripeptide. Saponification, oxidation, deprotection and HPLC purification, under the conditions as described in examples 1 (1 - n), gave HOOC-CH$_2$-D-Dpa-Pro-LysΨ[COCO]-OH (214 mg).

$R_t$(LC): 29.41 min, A 20 %, B 80 %, C 0 % to A 20 %, B 20 %, C 60 % in 40 min.

**EXAMPLE 10**

**HOOC-CH$_2$-D-p-Cl-Phe-Pro-LysΨ[COCO]-OH**

(a) **N-(t-Butyloxycarbonylmethyl)-N-Boc-D-p-Cl-Phe-OH**

According to analogous procedures as described in example 1, H-D-p-Cl-Phe-OH. HCl (10 g) was converted into N-(t-Butyloxycarbonylmethyl)-N-Boc-D-p-Cl-Phe-OH. Yield: 16.7 g.

$R_f = 0.27$, silica gel, ethyl acetate/methanol 9/1, v/v.

(b) **N-(t-Butyloxycarbonylmethyl)-N-Boc-D-p-Cl-Phe-OSu**

A solution of N-(t-Butyloxycarbonylmethyl)-N-Boc-D-p-Cl-Phe-OH (14.67 g) in 250 ml acetonitrile was treated with N-hydroxysuccinimide (4.11 g) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (6.86 g) overnight at room temperature. The reaction mixture was evaporated to dryness and the residue was dissolved in ethyl acetate. The organic phase was washed with water, dried over sodium sulfate and concentrated to afford 19.11 g active ester, which was directly used in the next step.
(c). \( N-(t-\text{Butyloxycarbonylmethyl})-N-\text{Boc-D-p-Cl-Phe-Pro-OH} \)

H-Pro-OH.HCl (10.79 g) was dissolved in 100 ml N,N-dimethyl formamide and 100 ml water. The pH of the reaction mixture was adjusted to 8 with a 1 N sodium hydroxide solution, whereafter N-(t-Butyloxycarbonylmethyl)-N-Boc-D-p-Cl-Phe-OSu (19.11 g), dissolved in 120 ml of N,N-dimethyl formamide, was added dropwise. The reaction was stirred overnight at room temperature at pH ≈ 8. The reaction mixture was cooled and adjusted to pH ≈ 2 with 1 N hydrochloric acid. The aqueous layer was extracted with dichloromethane (3x). The organic phase was washed with water, dried over sodium sulfate and evaporated in vacuo. Silicagel purification, using a gradient ethyl acetate/methanol 9/1 → 1/1 v/v, afforded 7.04 g of the desired dipeptide.

TLC: \( R_f = 0.24 \), silicagel, ethyl acetate/methanol 8/2 v/v.

(d). \( \text{HOOC-CH}_2\text{-D-p-Cl-Phe-Pro-Lys}^\Psi[\text{COCO}]\text{-OH} \)

N-(t-Butyloxycarbonylmethyl)-N-Boc-D-p-Cl-Phe-Pro-OH (692 mg) was coupled with H-Lys(Boc)^Ψ[CHOHCO]-OMe HCl (441 mg), under the conditions as described in example 1(k). Silica gel purification in dichloromethane/methanol 97/3 → 9/1 (v/v) afforded 835 mg of the fully protected tripeptide. Saponification, oxidation, deprotection and HPLC purification, under the conditions as described in examples 1(l - n), obtained \( \text{HOOC-CH}_2\text{-D-p-Cl-Phe-Pro-Lys}^\Psi[\text{COCO}]\text{-OH} \) (411 mg).

Rt(LC): 23.22 min, A 20 %, B 80 %, C 0 % to A 20 %, B 20 %, C 60 % in 40 min.

EXAMPLE 11

\( \text{HOOC-CH}_2\text{-D-Coa-Pro-Lys}^\Psi[\text{COCO}]\text{-OH} \)

(a). \( N-(t-\text{Butyloxycarbonylmethyl})-N-\text{Boc-D-Coa-Pro-OH} \)

According to the methods described in example 8(a), cyclooctylmethylbromide (27.49 g) and diethyl acetylaminalomalonate (29.10 g) were condensed to diethyl 2-acetylamino-2-
(cyclooctylmethyl)-malonate (19.60 g). Subsequent acid hydrolysis (example 8 (b)) afforded H-
D,L-Coa-OH.HCl (9.83 g). Esterification, alkylation, Boc protection, saponification and
subsequent coupling with H-Pro-OBzI, according to examples 1 (a - e), obtained N-(t-
Butyloxy carbonylmethyl)-N-Boc-D,L-Coa-Pro-OBzI (19.61 g). The benzyl ester was removed
by hydrogenolysis as in example 1 (f), whereafter the L/L-diastereomer was separated from the
D/L-diastereomer by crystallisation from diethyl ether to give the desired compound (9.34 g).
TLC: Rf = 0.1, silica gel, heptane/ethyl acetate = 3/1 v/v.

(b) HOOC-CH2-D-Coa-Pro-LysΨ[COCO]-OH

N-(t-Butyloxy carbonylmethyl)-N-Boc-D-Coa-Pro-OH (354 mg) was coupled with H-
Lys(Boc)Ψ[CHOHCO]-OMe HCl (243 mg), under the conditions as described in example 1.
Silica gel purification in heptane/ethylacetate 2/8 (v/v) afforded 471 mg of the fully protected
tripeptide. Saponification, oxidation, deprotection and HPLC purification, under the conditions
as described in examples 1, obtained HOOC-CH2-D-Coa-Pro-LysΨ[COCO]-OH (175 mg).
Rt(LC): 31.39 min, A 20 %, B 80 %, C 0 % to A 20 %, B 20 %, C 60 % in 40 min.

EXAMPLE 12

HOOC-CH2-D-p-CH3-Phe-Pro-LysΨ[COCO]-OH

(a) Fmoc-D-p-CH3-Phe-OMe

Fmoc-D-p-CH3-Phe-OH (5.0 g) was dissolved in dichloromethane (50 ml) and methanol (1 ml)
whereafter [2-(1H-Benzotriazol-1-yl)-1,1,3,3-tetramethylyuronium tetrafluoroborate] (4.4 g)
was added under nitrogen. The reaction mixture was adjusted to pH ≈ 8 with N,N-diisopropylethyl
amine and stirred overnight. The solvent was evaporated and the residue was
dissolved in ethyl acetate, washed with 5 % sodium hydrogen carbonate, water, aqueous
hydrogen chloride (0.1 N) and brine. The organic layer was dried over sodium sulfate and
evaporated in vacuo to afford Fmoc-D-p-CH3-Phe-OMe (5.6 g).
TLC: Rf = 0.55, silica gel, heptane/ethylacetate = 3/2 v/v.
(b). \textit{N-(t-Butyloxy carbonylmethyl)-N-Boc-D-p-CH$_3$-Phe-Pro-OH}

Fmoc-D-p-CH$_3$-Phe-OMe (5.6 g) was treated with piperidine (7 ml) and \textit{N,N-di}-

methyldimethylformamide (28 ml) for 1 hour at room temperature. The solvent was removed in vacuo
and the residue was dissolved in ethyl acetate. The organic layer was extracted with 0.1 N
aqueous hydrogen chloride (3x). The water layers were pooled and the pH of the solution was
adjusted to pH $\approx$ 9 with aqueous sodium hydroxide, followed by the extraction with
dichloromethane (3x). The organic layers were washed with water, dried over sodium sulfate
and evaporated in vacuo. The crude product was purified by silica gel chromatography, using
ethyl acetate/methanol 99/1 $\rightarrow$ 95/5 (v/v), to obtain H-D-p-CH$_3$-Phe-OMe (2.3 g). Alkylation
and Boc protection according to the methods described in examples 1 afforded \textit{N-(t-Butyloxy carbonylmethyl)-N-Boc-D-p-CH$_3$-Phe-OH} (4.3 g). Subsequent coupling with H-Pro-
OMe.HCl and saponification, according to the procedures described in examples 8, afforded the
title compound (3.6 g).

TLC: $R_f = 0.1$, silica gel, dichloromethane/methanol = 9/1 v/v.

c. \textit{HOOC-CH$_2$-D-p-CH$_3$-Phe-Pro-Lys$\Psi$(COCO)-OH}

\textit{N-(t-Butyloxy carbonylmethyl)-N-Boc-D-p-CH$_3$-Phe-Pro-OH} (397 mg) was coupled with H-
Lys(Boc)$\Psi$(CHOHCO)-OMe.HCl (264 mg), under the conditions as described in example 1.
Silica gel purification in dichloromethane/methanol 97/3 $\rightarrow$ 9/1 (v/v) afforded 450 mg of the
fully protected tripeptide. Saponification, oxidation, deprotection and HPLC purification, under
the conditions as described in examples 1, obtained \textit{HOOC-CH$_2$-D-p-CH$_3$-Phe-Pro-
Lys$\Psi$(COCO)-OH} (197 mg)

\textit{Rt(LC): 22.36 min, A 20%, B 80%, C 0% to A 20%, B 20%, C 60% in 40 min.}

\textsc{Example 13}

\textit{HOOC-CH$_2$-D/L-m,p-di-Cl-Phe-Pro-Lys$\Psi$(COCO)-OH}

(a). \textit{N-(t-Butyloxy carbonylmethyl)-N-Boc-D/L-m,p-di-Cl-Phe-Pro-OH}
H-D/L-m,p-di-Cl-Phe-OH was prepared according to the methods as described in examples 8, starting from 3,4-dichlorobenzyl chloride. Esterification, alkylation, Boc protection, saponification and coupling with H-Pro-OMe.HCl were performed according to the procedures described in examples 1. Subsequent saponification of the dipeptide (2.2 g) using sodium hydroxide (see example 8) afforded N-(t-Butyloxycarbonylmethyl)-N-Boc-D/L-m,p-di-Cl-Phe-Pro-OH (2.2 g)

TLC: Rf = 0.52 and 0.64, silica gel, ethyl acetate/pyridine/acetic acid/water = 163/20/6/11 v/v/v/v.

(b). HOOC-CH$_2$D/L-m,p-di-Cl-Phe-Pro-Lys$\Psi$(COCO)-OH

N-(t-Butyloxycarbonylmethyl)-N-Boc-D/L-m,p-di-Cl-Phe-Pro-OH (402 mg) was coupled with H-Lys(Boc)$\Psi$(CHOHCO)-OMe.HCl (243 mg), under the conditions as described in example 1. Silica gel purification in heptane/ethyl acetate 1/1 → 1/4 (v/v) afforded 545 mg of the fully protected tripeptide. Saponification, oxidation, deprotection and HPLC purification, under the conditions as described in examples 1, obtained HOOC-CH$_2$D/L-m,p-di-Cl-Phe-Pro-Lys$\Psi$(COCO)-OH (125 mg) as a mixture of diastereomers and rotamers.

Rt(LC): 22.96, 24.20, 25.91 and 26.33 min, A 20 %, B 80 %, C 0 % to A 20 %, B 20 %, C 60 % in 40 min.

EXAMPLE 14

HOOC-CH$_2$CH$_2$-D-Cha-Pro-Lys$_\Psi$(COCO)O$_2$H

(a). N-(2-(t-Butyloxycarbonyl)ethyl)-N-Boc-D-Cha-OMe

To H-D-Cha-OMe.HCl (711 g) in acetonitrile (25 mL) under a nitrogen atmosphere were added N,N-diisopropylethylamine (2.6 mL) and tert-butyl acrylate (25 mL) and this stirred suspension was heated at 40 °C keeping the pH of the reaction mixture at eight by addition of N,N-diisopropylethylamine. After four days additional tert-butyl acrylate (5 mL) was added and the reaction mixture was heated at 40 °C for an additional three days keeping the pH at eight.
The reaction mixture was concentrated under reduced pressure. The residue was dissolved in ethyl acetate, washed with water and brine, dried over sodium sulfate and concentrated. The residue was dissolved in N,N-dimethylformamide (10mL), di-tert-butyl dicarbonate (7.86 g) was added and the pH of the solution was adjusted to eight and maintained at this pH using N,N-diisopropylethylamine. After stirring for 30 hours additional di-tert-butyl dicarbonate (1.0 g) was added and the reaction mixture was stirred at room temperature for an additional day. The reaction mixture was concentrated under reduced pressure. The residue was dissolved in ethyl acetate, washed with water and brine, dried over sodium sulfate and concentrated. The residue was purified using column chromatography (silica gel, ethyl acetate/heptane = 1/5 v/v) to yield N-(2-(t-butyloxycarbonyl)ethyl)-N-Boc-D-Cha-OMe (8.78 g).

TLC: R_f = 0.7, silica gel, ethyl acetate/heptane=1/2 v/v.

(b) N-(2-(t-butyloxycarbonyl)ethyl)-N-Boc-D-Cha-OH

To stirred solution of (N-(2-(t-butyloxycarbonyl)ethyl)-N-Boc-D-Cha-OMe (10.0 g) in dioxane (75 mL) and water (10 mL) at room temperature was added 2N sodium hydroxide at such rate that the pH of the reaction mixture was kept at 12.5. After 6.5 hours the reaction mixture was neutralised and filtered. The filtrate was concentrated to half its volume, cooled on an ice bath, made acid (pH 3) using 4N hydrochloric acid and extracted with ethyl acetate. The ethyl acetate extract was washed with cold hydrochloric acid (pH 3). The aqueous layers were washed with ethyl acetate and the combined ethyl acetate extracts were dried over sodium sulphate and concentrated. The residue was purified using column chromatography (silica gel, ethyl acetate/heptane = 1/1 v/v) to yield (tBuOOC-CH₂CH₂)(Boc)-D-Cha-OH (4.31 g).

TLC: R_f = 0.1, silica gel, ethyl acetate/heptanes=1/5 v/v.

(c) N-(2-(t-butyloxycarbonyl)ethyl)-N-Boc-D-Cha-Pro-Lys(Boc)Ψ[COCO]-OMe

N-(2-(t-butyloxycarbonyl)ethyl)-N-Boc-D-Cha-OH (3.14 g) was coupled with H-Pro-OBzl.HCl and hydrogenated using procedures described in example 1 to give N-(2-(t-butyloxycarbonyl)ethyl)-N-Boc-D-Cha-Pro-OH (3.96 g). This dipeptide (0.46 g) was coupled with H-Lys(Boc)Ψ[CHOHCO]-OMe according to the procedures described in example 1 to
give N-(2-(t-butyloxy carbonyl)ethyl)-N-Boc-D-Cha-Pro-Lys(Boc)Ψ[CHOHCO]-OMe. N-(2-
(t-Butyloxy carbonyl)ethyl)-N-Boc-D-Cha-Pro-Lys(Boc)Ψ[CHOHCO]-OMe was used in the
Dess Martin oxidation as described in example 1 to yield the title compound (0.55 g)
TLC: Rf = 0.6, silica gel, ethyl acetate.

5 (d) HOOC-CH_{2}CH_{2}-D-Cha-Pro-LysΨ[COCO]OH

To a stirred solution of N-(2-(t-butyloxy carbonyl)ethyl)-N-Boc-D-Cha-Pro-
Lys(Boc)Ψ[COCO]-OMe (0.55 g) in dichloromethane (6 mL) at room temperature was added
trifluoroacetic acid (2 mL). After six hours the solution was concentrated under reduced
pressure and once coevaporated with water. Water (17 mL) and methanol (1 mL) were added
to the residue, the resulting suspension was filtered and 36 - 38% hydrochloric acid (1 mL) was
added to the filtrate. After one week at room temperature the solution was concentrated under
reduced pressure, dissolved in water and charged onto a preparative HPLC DeltaPak RP-C_{18}
using a gradient elution system of 20% A/80% B to 20% A/50% B/30% C over 40 min at a
flow rate of 80 mL. Yield: 277 mg of HOOC-CH_{2}CH_{2}-D-Cha-Pro-LysΨ[COCO]-OH.
Rt(LC): 22.2 min, A 20 %, B 80 %, C 0 % to A 20 %, B 20 %, C 60 % in 40 min.

EXAMPLE 15

HOOC-CH_{2}-D-Chg-Pro-LysΨ[COCO]-OH

(a). H-D-Chg-OMe HCl

H-D-Chg-OMe HCl was obtained by reacting H-D-Chg-OH HCl (10.0 g) with thionylchloride
(7.5 mL) and methanol (100 mL) according to the procedure described in example 1.
Yield: 9.66 g.
TLC: Rf = 0.58, silica gel, ethyl acetate/pyridine/acetic acid/water = 88/31/18/7 v/v/v/v.
(b) \textbf{N-}(t-\text{Butyloxy carbonylmethyl})-\textbf{N}-\text{Boc-}D-\text{Chg-OMe}

\textbf{N-}(t-\text{Butyloxy carbonylmethyl})-D-\text{Chg-OMe} \text{ was obtained by reacting } H-\text{D-Chg-OMe}\cdot\text{HCl} (9.66 \text{ g}) \text{ with } t-\text{butyl-bromo-acetate} (9.10 \text{ g}) \text{ for } 4 \text{ days according to the procedure described in example } 1, \text{ washing the crude organic layer with water, } 5\% \text{ sodium hydrogen carbonate and brine, yielded } \textbf{N-}(t-\text{butyloxy carbonylmethyl})-D-\text{Chg-OMe} (14.04 \text{ g}) \text{ as a crude product. The title compound was obtained by reacting crude } \textbf{N-}(t-\text{butyloxy carbonylmethyl})-D-\text{Chg-OMe} (14.04 \text{ g}) \text{ with } \text{di-}t-\text{butyl dicarbonate} (10.18 \text{ g}) \text{ in } 65 \text{ hours according to the procedure described in example } 1, \text{ The crude organic layer was washed with water, brine, dried on magnesium sulphate and concentrated in vacuo. The residue was chromatographed on silica in heptane/ethyl acetate = 9/1 (v/v) as eluent. The fractions containing } \textbf{N-}(t-\text{butyloxy carbonylmethyl})-\textbf{N}-\text{Boc-}D-\text{Chg-OMe} \text{ were pooled and evaporated. Yield: } 15.69 \text{ g}. \text{TLC: } R_f = 0.72, \text{ silica gel, heptane/ethyl acetate = 6/4 v/v.}

(c) \textbf{N-}(t-\text{Butyloxy carbonylmethyl})-\textbf{N}-\text{Boc-}D-\text{Chg-OH}

\text{To a solution of } \textbf{N-}(t-\text{butyloxy carbonylmethyl})-\textbf{N}-\text{Boc-}D-\text{Chg-OMe} (15.69 \text{ g}) \text{ in tetra-hydrofuran (140 mL) was added a suspension of lithium hydroxide (3.42 g) in water (60 mL) and stirred for 20 hours at room temperature. After diluting with icewater and acidification with 2N hydrochloric acid to pH 2, the mixture was extracted twice with dichloromethane. The organic layer was washed with water, brine, dried on magnesium sulphate and evaporated in vacuo. Yield: } 14.91 \text{ g}. \text{TLC: } R_f = 0.55, \text{ dichloromethane/methanol = 9/1 v/v.}

(d) \textbf{N-}(t-\text{Butyloxy carbonylmethyl})-\textbf{N}-\text{Boc-}D-\text{Chg-Pro-OH}

\textbf{N-}(t-\text{Butyloxy carbonylmethyl})-\textbf{N}-\text{Boc-}D-\text{Chg-OH} (2.51 \text{ g}) \text{ was coupled with } H-\text{Pro-OBzl}\cdot\text{HCl} (1.80 \text{ g}) \text{ and hydrogenated according to the procedures described in example } 1, \text{ yielding } \textbf{N-}(t-\text{butyloxy carbonylmethyl})-\textbf{N}-\text{Boc-}D-\text{Chg-Pro-OH} (2.57 \text{ g}).
(e) HOOC-CH$_2$-D-Chg-Pro-Lys$\Psi$(COCO)-OH

This dipeptide (248 mg) was coupled with H-Lys(Boc)$\Psi$(CHOHCO)-OMe (190 mg), saponificated, oxidized, deprotected and purified according to the procedures described in example 1, yielding HOOC-CH$_2$-D-Chg-Pro-Lys$\Psi$(COCO)-OH (98 mg).

Rt(LC): 13.04 min., A 20 %, B 80 %, C 0 % to A 20 %, B 24 %, C 56 % in 40 min.

EXAMPLE 16

HOOC-CH$_2$-D-2-Nal-Pro-Lys$\Psi$(COCO)-OH

(a) H-D-2-Nal-OMe TFA

N-Boc-D-2-Nal-OMe was obtained by reacting N-Boc-D-2-Nal-OH (7.5 g) with 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (7.63 g) in dichloromethane / methanol 9/1 v/v according to the procedure described in example 1. Yield: 7.2 g. This methylester (7.2 g) was deprotected with trifluoroacetic acid in 1 hour at room temperature after which the reaction mixture was evaporated in vacuo, yielding H-D-2-Nal-OMe. trifluoroacetic acid (5.01 g).

TLC: $R_f = 0.65$, silica gel, ethyl acetate/pyridine/acetic acid/water = 63/20/6/11 v/v/v/v.

(b) N-(t-Butyloxycarbonylmethyl)-N-Boc-D-2-Nal-OMe

N-(t-Butyloxycarbonylmethyl)-D-2-Nal-OMe was obtained by reacting H-D-2-Nal-OMe.trifluoroacetic acid (5.01 g) with t-butylbromoacetate (4.26 g) according to the procedure described in example 1, washing the crude organic layer with water, 5% sodium hydrogen carbonate and brine, yielding N-(t-butyloxycarbonylmethyl)-D-2-Nal-OMe (8.52 g) as a crude product. The title compound was obtained by reacting crude N-(t-butyloxycarbonylmethyl)-D-2-Nal-OMe (8.52 g) with di-t-butyl dicarbonate (4.77 g) in acetonitril according to the procedure described in example 1. The crude organic layer was washed with water, brine, dried on magnesium sulphate and concentrated in vacuo. The residue was chromatographed on silica
in heptane/ethyl acetate = 9/1 (v/v) as eluent. The fractions containing N-(t-butyloxy-carbonylmethyl)-N-Boc-D-2-Nal-OMe were pooled and evaporated. Yield: 6.7 g.

TLC: Rf = 0.71, silica gel, heptane/ethyl acetate = 1/1 v/v.

(c) N-(t-Butyloxy carbonylmethyl)-N-Boc-D-2-Nal-Pro-OH

N-(t-Butyloxy carbonylmethyl)-N-Boc-D-2-Nal-Pro-OH was obtained by saponification of N-(t-butyloxy carbonylmethyl)-N-Boc-D-2-Nal-OMe (6.7 g), coupling with H-Pro-OBzl HCl (3.57 g) and hydrogenating according to the procedures described in example 1. Yield: 7.3 g.

(d) HOOC-CH$_2$-D-2-Nal-Pro-Lys[COCO]-OH

The dipeptide described in 16 (c) (327 mg) was coupled with H-Lys(Boc)[CHOHCO]-OMe (193 mg) in 4 hours and the crude product was saponified, oxidized, deprotected and purified according to the procedures described in example 1, yielding HOOC-CH$_2$-D-2-Nal-Pro-Lys[COCO]-OH (152 mg).

Rt(LC): 25.90 min., A 20%, B 80%, C 0% to A 20%, B 20%, C 60% in 40 min.

EXAMPLE 17

HOOC-CH$_2$-D-Nle-Pro-Lys[COCO]-OH

(a) N-(t-Butyloxy carbonylmethyl)-N-Boc-D-Nle-Pro-OH

Starting from H-D-Nle-OH (2.9 g), the title compound (6.0 g) was prepared using the methodologies described in example 1.

TLC: Rf = 0.65, silica gel, ethyl acetate/pyridine/acetic acid/water = 363/20/6/11 v/v/v/v
(b) \( \text{HOOC-CH}_2\text{-D-Nle-Pro-Lys}'[\text{COCO}]\text{-OH} \)

\( \text{N-}(\text{t-Butyloxycarbonylmethyl})\text{-N-Boc-D-Nle-Pro-OH} \) (377 mg) was coupled with \( \text{H-Lys(Boc)}'[\text{CHOHCO}]\text{-OMe HCl} \) (321 mg), under the conditions as described in example 1. Silica gel purification in dichloromethane/methanol 99/1 → 95/5 (v/v) afforded 600 mg of the fully protected tripeptide. Saponification, oxidation, deprotection and HPLC purification, under the conditions as described in examples 1, resulted in \( \text{HOOC-CH}_2\text{-D-Nle-Pro-Lys}'[\text{COCO}]\text{-OH} \) (213 mg).

\( \text{Rt(LC): 14.95 min, A 20 \%, B 80 \%, C 0 \% to A 20 \%, B 20 \%, C 60 \% in 40 min.} \)

**EXAMPLE 18**

**\( \text{HOOC-CH}_2\text{-D-p-OEt-Phe-Pro-Lys}'[\text{COCO}]\text{-OH} \)**

(a) \( \text{H-D-p-OEt-Phe-OMe} \)

\( \text{Boc-D-p-OEt-Phe-OH} \) (5 g) was dissolved in 80 ml dichloromethane/methanol 9/1 v/v and TBTU (5.6 g) was added and the pH was adjusted to 8.5 with triethyl amine. Next, the solution was stirred for 1 h at room temperature, washed with 1 N HCl, water, 5 \% sodium hydrogencarbonate and water, dried on sodium sulfate and evaporated to dryness. This yielded 5.6 g of the methyl ester. This intermediate was dissolved in 100 ml dichloromethane/trifluoroacetic acid 1/1 v/v and stirred for 1 h at room temperature, concentrated to dryness, yielding 5.3 g of an oil (trifluoroacetate salt).

\( \text{TLC: R}_f=0.21, \text{silica gel, dichloromethane/methanol 95/5 v/v.} \)

(b) \( \text{HOOC-CH}_2\text{-D-p-OEt-Phe-Pro-Lys}'[\text{COCO}]\text{-OH} \)

Starting with \( \text{H-D-p-OEt-Phe-OMe.TFA} \), a synthetic route is followed similar to example 1, thereby producing the title compound.
EXAMPLE 19

HOOC-CH$_2$-D-Aca-Pro-LysΨ[COCO]-OH

The tripeptide was prepared similar to the route followed for example 1 starting from 2-Aminocaprylic acid. Yield: 206 mg.

R$_d$(LC): 23.95 min.; 20% A/80% B to 20% A/20% B/60% C in 40 min.

EXAMPLE 20

HOOC-CH$_2$-D-Cha-cisEthylPro-LysΨ[COCO]-OH

N-(t-Butyloxycarbonylmethyl)-N-Boc-D-Cha-cisEthylPro-OH was prepared according a similar manner as described in example 6. Next the tripeptide was prepared similar to the route followed for example 1. Yield: 130 mg.

R$_d$(LC): 32.06 min.; 20% A/80% B to 20% A/20% B/60% C in 40 min.

EXAMPLE 21

HOOC-CH$_2$-D-Cha-Azt-LysΨ[COCO]-OH

(a) N-(t-Butyloxycarbonyl)-Azt-OH

L-azetidine-2-carboxylic acid (2.44 g) was dissolved in 75 ml t-butanol/water=2/1 v/v. After addition of 4 ml 6M NaOH-solution di-t-butyl dicarbonate (5.79 g) was added and the reaction was stirred for 2 h at room temperature. The mixture was diluted with 250 ml of water and extracted with heptane (3 times). After acidification, the mixture was extracted with dichloromethane. The organic layer was washed with water and was dried on sodium sulfate. The filtrate was evaporated and yielded 5 g of N-(t-Butyloxycarbonyl)-Azt-OH.

TLC: R$_f$=0.50, silica gel, ethyl acetate/pyridine/acetic acid/water=163/20/6/11 v/v/v/v.
(b) \textit{N-}(t-\text{Butyloxy carbonyl})-\text{Azt-OBzl}

\text{N-}(t-\text{Butyloxycarbonyl})-\text{Azt-OH} (4.6 \text{ g}) was dissolved in dichloromethane (50 \text{ ml}), 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (23.6 \text{ g}) and benzylalcohol (2.47 \text{ g}) were added and the pH of the solution was adjusted to 8 by addition of triethylamine. The reaction mixture was stirred for 1 \text{ h} at room temperature. The mixture was washed successively with cold 1N hydrogen chloride solution, water, 5\% sodium hydrogen carbonate, and water and dried over sodium sulfate. The filtrate was evaporated and the residue was chromatographed on silica gel in ethylacetate/heptane = 1/3 \text{ v/v} as eluent. The fractions containing \textit{N-}(t-\text{Butyloxycarbonyl})-\text{Azt-OBzl} were pooled and evaporated. Yield: 6 \text{ g}. TLC: \text{R}_f= 0.95, \text{silica gel, ethyl acetate/pyridine/acetic acid/water}=163/20/6/11 \text{ v/v/v/v}.

(c) \textit{HClH-}\text{Azt-OBzl}

\text{N-}(t-\text{Butyloxycarbonyl})-\text{Azt-OBzl} (6 \text{ g}) was dissolved in 60 \text{ ml} 3M \text{HCl/dioxane} and stirred for 1 \text{ h} at room temperature. The reaction mixture was evaporated to dryness yielding 5.26 \text{ g} of a white solid. TLC: \text{R}_f= 0.50, \text{silica gel, ethyl acetate/pyridine/acetic acid/water}=63/20/6/11 \text{ v/v/v/v}.

(d) \textit{HOOC-CH}_2-\text{D-Cha-Azt-Lys}\Psi[\text{COCO}]-\text{OH}

The tripeptide was prepared according to a similar route as described in example 1.
Yield: 120 \text{ mg}.
\text{R}_t(\text{LC}) : 21.09 \text{ min} ; 20\% A/80\% B \text{ to } 20\% A/20\% B/60\% C \text{ in } 40 \text{ min}.

\textbf{EXAMPLE 22}

\textbf{HOOC-CH}_2-\text{D-Cha-Ohi-Lys}\Psi[\text{COCO}]-\text{OH}
N-(t-Butyloxycarbonylmethyl)-N-Boc-D-Cha-OH-OH was prepared according a similar manner as described in example 5. Next the tripeptide was prepared similar to the route followed for example 1. Yield: 147 mg.

Rt(LC): 33.03 min.; 20% A/80% B to 20% A/20% B/60% C in 40 min.

EXAMPLE 23

HOOC-CH₂-D-Cha-DehydroPro-LysΨ[CO]CO]·OH

(a). N-(t-Butyloxycarbonylmethyl)-N-Boc-D-Cha-DehydroPro-OH

The active-ester coupling between N-(t-Butyloxycarbonylmethyl)-N-Boc-D-Cha-OH and H-3,4-DehydroPro-OMe.HCl and the subsequent saponification to yield 2.58 g N-(t-Butyloxycarbonylmethyl)-N-Boc-D-Cha-DehydroPro-OH were done according to the procedures described in example 5.

TLC: Rₜ=0.74, silica gel, ethyl acetate/pyridine/acetic acid/water = 163/20/6/11 v/v/v/v.

(b). HOOC-CH₂-D-Cha-DehydroPro-LysΨ[CO]CO]·OH

The active-ester coupling between N-(t-Butyloxycarbonylmethyl)-N-Boc-D-Cha-DehydroPro-OH and H-Lys(Boc)Ψ[CHOHCO]-OMe.HCl, the saponification, the Dess-Martin oxidation, the deprotection and the purification were done according to the procedures described in example 1, yielding 132 mg HOOC-CH₂-D-Cha-DehydroPro-LysΨ[CO]CO]·OH.

Rt(LC): 23.33 min, 20% A/80% B to 20% A/20% B/60% C in 40 min.

EXAMPLE 24

HOOC-CH₂-D-Cha-Val-LysΨ[CO]CO]·OH

(a). N-(t-Butyloxycarbonylmethyl)-N-Boc-D-Cha-Val-OH
The active-ester coupling between N-(t-Butyloxy carbonylmethyl)-N-Boc-D-Cha-OH and H-Val-OMe·HCl and the subsequent saponification to yield 2.04 g N-(t-Butyloxy carbonylmethyl)-N-Boc-D-Cha-Val-OH were done according to the procedures described in example 5.

TLC: R_t=0.81, silica gel, ethyl acetate/pyridine/acetic acid/water = 163/20/6/11 v/v/v/v.

(b) HOOC-CH_2-D-Cha-Val-Lys\Psi[COCO]-OH

The active-ester coupling between N-(t-Butyloxy carbonylmethyl)-N-Boc-D-Cha-Val-OH and H-Lys(Boc)\Psi[CHOHCO]-OMe·HCl, the saponification, the Dess-Martin oxidation, the deprotection and the purification were done according to the procedures described in example 1, yielding 61 mg HOOC-CH_2-D-Cha-Val-Lys\Psi[COCO]-OH.

R_t(LC): 22.99 min, 20% A/80% B to 20% A/40% B/40% C in 40 min.

**EXAMPLE 25**

**HOOC-CH_2-D-Cha-Pec-Lys\Psi[COCO]-OH**

(a) H-Pec-OBzl·HCl

The synthesis of H-Pec-OBzl·HCl starting with H-Pec-OH was done according to the procedures described in example 21, yielding 1.74 g H-Pec-OBzl·HCl.

TLC: R_t=0.31, silica gel, ethyl acetate/pyridine/acetic acid/water = 163/20/6/11 v/v/v/v.

(b) N-(t-Butyloxy carbonylmethyl)-N-Boc-D-Cha-Pec-OH

The active-ester coupling between N-(t-Butyloxy carbonylmethyl)-N-Boc-D-Cha-OH and H-Pec-OBzl·HCl and the subsequent debenzylation to yield 2.04 g N-(t-Butyloxy carbonylmethyl)-N-Boc-D-Cha-Pec-OH were done according to the procedures described in example 1.

TLC: R_t=0.36, silica gel, dichloromethane/methanol 9/1 v/v.
(c) HOOC-CH$_2$-D-Cha-Pec-Lys'$\Psi$(COCO)-OH

The active-ester coupling between N-(t-Butyloxycarbonylmethyl)-N-Boc-D-Cha-Pec-OH and H-Lys(Boc)'$\Psi$[CHOHCO]-OMe·HCl, the saponification, the Dess-Martin oxidation, the deprotection and the purification were done according to the procedures described in example 1, yielding HOOC-CH$_2$-D-Cha-Pec-Lys'$\Psi$(COCO)-OH.

Yield of two diastereomers:
58 mg. Rt(LC): 23.02 min, 20% A/80% B to 20% A/20% B/60% C in 40 min.
104 mg. Rt(LC): 27.13 min, 20% A/80% B to 20% A/20% B/60% C in 40 min.

EXAMPLE 26

HOOC-CH$_2$-D-Cha-3,3-Dmp-Lys'$\Psi$(COCO)-OH

(a) H-3,3-Dmp-OMe·HCl

H-3,3-Dmp-OMe·HCl was synthesized from H-3,3-Dmp-OH (US 4,060,603) according to the procedures described in example 1, yielding 590 mg H-3,3-Dmp-OMe·HCl.
TLC: R$_t$=0.60, silica gel, n-butanol/pyridine/acetic acid/water = 4/1/1/2 v/v/v/v.

(b) N-(t-Butyloxycarbonylmethyl)-N-Boc-D-Cha-3,3-Dmp-OH

The active-ester coupling between N-(t-Butyloxycarbonylmethyl)-N-Boc-D-Cha-OH and H-3,3-Dmp-OMe·HCl and the subsequent saponification to yield 2.58 g N-(t-Butyloxycarbonylmethyl)-N-Boc-D-Cha-3,3-Dmp-OH were done according to the procedures described in example 5.
TLC: R$_t$=0.74, silica gel, ethyl acetate/pyridine/acetic acid/water = 163/20/6/11 v/v/v/v.
(c) **HOOC-CH$_2$-D-Cha-3,3-Dmp-Lys$\Psi$(COCO)-OH**

The active-ester coupling between N-{(t-Butyloxycarbonylmethyl)}-N-Boc-D-Cha-3,3-Dmp-OH and H-Lys(Boc)$\Psi$(CHOHCO)-OMe.HCl, the saponification, the Dess-Martin oxidation, the deprotection and the purification were done according to the procedures described in example 1, yielding 119 mg HOOC-CH$_2$-D-Cha-3,3-Dmp-Lys$\Psi$(COCO)-OH.

Rt(LC): 29.32 min, 20% A/80% B to 20% A/20% B/60% C in 40 min.

**EXAMPLE 27**

**HOOC-CH$_2$-D-Cha-Aib-Lys$\Psi$(COCO)-OH**

(a). **H-Aib-OMe.HCl**

The synthesis of H-Aib-OMe.HCl starting with H-Aib-OH was done according to the procedures described in example 1, yielding 1.89 g H-Aib-OMe.HCl.

TLC: $R_f=0.58$, silica gel, n-butanol/pyridine/acetic acid/water = 4/1/1/2 v/v/v/v.

(b). **N-{(t-Butyloxycarbonylmethyl)}-N-Boc-D-Cha-Aib-OH**

The active-ester coupling between N-{(t-Butyloxycarbonylmethyl)}-N-Boc-D-Cha-OH and H-Aib-OMe.HCl and the subsequent saponification to yield 2.58 g N-{(t-Butyloxycarbonylmethyl)}-N-Boc-D-Cha-Aib-OH were done according to the procedures described in example 5.

TLC: $R_f=0.79$, silica gel, ethyl acetate/pyridine/acetic acid/water = 163/20/6/11 v/v/v/v.

(c). **HOOC-CH$_2$-D-Cha-Aib-Lys$\Psi$(COCO)-OH**

The active-ester coupling between N-{(t-Butyloxycarbonylmethyl)}-N-Boc-D-Cha-Aib-OH and H-Lys(Boc)$\Psi$(CHOHCO)-OMe.HCl, the saponification, the Dess-Martin oxidation, the deprotection and the purification were done according to the procedures described in example 1, yielding 104 mg HOOC-CH$_2$-D-Cha-Aib-Lys$\Psi$(COCO)-OH.
Rt(LC): 22.82 min, 20% A/80% B to 20% A/20% B/60% C in 40 min.

EXAMPLE 28

**HOOC-CH₂-D-Chg-Azt-LysΨ[COCO]-OH**

N-(t-Butyloxy carbonylmethyl)-N-Boc-D-Chg-OH (2.58 g) (according to example 15) was coupled with H-Azt-OBzl.HCl (2.97 g) (according to example 21) according to the procedure described in example 1 yielding N-(t-butyloxy carbonylmethyl)-N-Boc-D-Chg-Azt-OBzl (1.02 g). This dipeptide (253 mg) was hydrogenated, coupled with H-Lys(Boc)Ψ[CHOHCO]-OMe (161 mg), saponified, oxidized, deprotected and purified according to the procedures described in example 1, yielding HOOC-CH₂-D-Chg-Azt-LysΨ[COCO]-OH (65 mg).

Rt(LC): 14.34 min, A 20 %, B 80 %, C 0 % to A 20 %, B 20 %, C 60 % in 40 min.

EXAMPLE 29

**HOOC-CH₂-D-p-OME-Phe-Azt-LysΨ[COCO]OH**

(a) **N-(t-Butyloxy carbonylmethyl)-N-Boc-D-p-OME-Phe-Azt-Lys(Boc)Ψ[COCO]-OH**

N-(t-Butyloxy carbonylmethyl)-N-Boc-D-p-OME-Phe-OH (3.02 g) was coupled with H-Azt-OBzl.TFA and hydrogenated using procedures described in example 1 to give N-(t-butyloxy carbonylmethyl)-N-Boc-D-p-OME-Phe-Azt-OH (1.48 g).

TLC: Rf = 0.37, silica gel, heptane/ethyl acetate = 6/4 v/v.

This dipeptide (297 mg) was coupled with H-Lys(Boc)Ψ[CHOHCO]-OMe, saponified and oxidized according to the procedures described in example 1 to yield the title compound (308 mg).

TLC: Rf = 0.60, silica gel, ethyl acetate/pyridine/acetic acid/water = 63/20/6/11 v/v/v/v.
(b). HOOC-CH₂-D-p-OMe-Phe-Azt-Lysψ[COCO]OH

N-(t-Butyloxy carbonylmethyl)-N-Boc-D-p-OMe-Phe-Azt-Lys(Boc)ψ[COCO]-OH (308 mg, crude) was treated with 50% trifluoroacetic acid/dichloromethane (3 ml) for 4 h at room temperature. The reaction mixture was concentrated in vacuo, coevaporated with toluene and the residue dissolved in water and directly charged onto a preparative HPLC DeltaPak RP-C₁₈ using a gradient elution system of 20% A/80% B to 20% A/65% B/15% C over 45 min at a flow rate of 70 ml/min at 45°C.

Yield: 118 mg of HOOC-CH₂-D-p-OMe-Phe-Azt-Lysψ[COCO]OH as a mixture of diastereomers.

R₅(LC): 16.1 min. and 16.7 min.; 20% A/80% B to 20% A/20% B/60% C in 40 min.

EXAMPLE 30

HOOC-CH₂-D-p-OMe-Phe-Val-Lysψ[COCO]-OH

(a). N-(t-Butyloxy carbonylmethyl)-N-Boc-D-p-OMe-Phe-Val-OH

To a solution of N-(t-butyloxy carbonylmethyl)-N-Boc-D-p-OMe-Phe-Val-OMe (1.86 g) (prepared according to example 1) in 45 mL of dioxane/water 2/1 v/v was added lithium hydride (600 mg) and stirred for 2.5 hours at room temperature. After diluting with ice-water and acidification with 2N hydrochloric acid to pH=2, the mixture was extracted twice with ethyl acetate. The organic layer was washed with water, brine, dried on magnesium sulphate and evaporated in vacuo. The residue was chromatographed on silica gel in dichloromethane/methanol 9/1 v/v as eluent. The fractions containing the desired compound were pooled and evaporated in vacuo. Yield: 1.77 g.

TLC: R₅ = 0.58, dichloromethane/methanol = 9/1 v/v.
(b) HOOC-CH₂-D-p-OMe-Phe-Val-LysΨ[COCO]-OH

N-(t-Butyloxycarbonylmethyl)-N-Boc-D-p-OMe-Phe-Val-OH (390 mg) was coupled with H-Lys(Boc)Ψ[CHOHCO]-OMe (192 mg), saponificated, oxidized, deprotected and purified according to the procedures described in example 1, yielding HOOC-CH₂-D-p-OMe-Phe-Val-LysΨ[COCO]-OH (50 mg).
Rₓ(LC) : 21.9 min.; 20% A/80% B to 20% A/20% B/60% C in 40 min.

EXAMPLE 31

HOOC-CH₂-D-Cha-Pro-LysΨ[COCO]-OiP

(a) Cbz-Lys(Boc)Ψ[CHOHCO]-OiP

Cbz-Lys(Boc)Ψ[CHOHCO]-OMe (6.1 g) was dissolved in 120 ml of 3N hydrogen chloride in 2-propanol and stirred during 4 days at 40 °C. The reaction was evaporated to dryness and coevaporated three times with 2-propanol to yield Cbz-LysΨ[CHOHCO]-OiP. This product was dissolved in 125 ml dry dichloromethane and 16 ml 2-propanol and di-tert-butyl dicarbonate (4.55 g) was added. The pH of the solution was adjusted and maintained at 8 with triethylamine and the reaction was stirred for 3 days at room temperature. Water was added and the organic layer was washed twice with water and dried. The residue was purified by chromatography on silica gel (eluent: gradient of heptane/ethyl acetate 7/3 v/v to 6/4 v/v) to yield Cbz-Lys(Boc)Ψ[CHOHCO]-OiP (2.9 g).
TLC: Rₓ= 0.25, silica gel, heptane/ethyl acetate= 6/4 v/v.

(b) H-Lys(Boc)Ψ[CHOHCO]-OiP

To a solution of Cbz-Lys(Boc)Ψ[CHOHCO]-OiP (880 mg) in DMF (10 ml) were added 10% palladium on activated carbon (100 mg) and 2N HCl (1.0 ml) and this suspension was hydrogenated at atmospheric pressure for 2 hours at room temperature. The palladium catalyst
was removed by filtration and the filtrate was concentrated in vacuo to yield H-Lys(Boc)ψ[CHOHCO]-OIP·HCl quantitatively.

TLC: Rf = 0.71, silica gel, ethyl acetate/pyridine/acetic acid/water = 88/31/18/7 v/v/v/v.

(c). **N-(t-Butyloxy carbonylmethyl)-N-Boc-D-Cha-Pro-Lys(Boc)ψ{COCO}-OIP**

N-(t-Butyloxy carbonylmethyl)-N-Boc-D-Cha-Pro-OH (275 mg) was coupled with H-Lys(Boc)ψ[CHOHCO]-OIP according to the procedures described in example 1 to give N-(t-butyloxy carbonylmethyl)-N-Boc-D-Cha-Pro-Lys(Boc)ψ[CHOHCO]-OIP (270 mg).

N-(t-Butyloxy carbonylmethyl)-N-Boc-D-Cha-Pro-Lys(Boc)ψ[CHOHCO]-OIP was used in the Dess Martin oxidation as described in example 3 to yield the title compound (258 mg).

TLC: Rf = 0.26, silica gel, heptane/ethyl acetate = 1/1 v/v.

(d). **HOOC-CH₂-D-Cha-Pro-Lysψ{COCO}-OIP**

N-(t-Butyloxy carbonylmethyl)-N-Boc-D-Cha-Pro-Lys(Boc)ψ[COOC]-OIP (258 mg, crude) was deprotected and purified using the procedure described in example 29 to give HOOC-CH₂-D-Cha-Pro-Lysψ{COCO}-OIP (75 mg).

Rₜ(LC) : 30.5 min.; 20% A/80% B to 20% A/20% B/60% C in 40 min.

**EXAMPLE 32**

**HOOC-CH₂-D-p-OMe-Phe-Pro-Lysψ{COCO}-OIP**

The procedure used to prepare example 31 was also used to prepare HOOC-CH₂-D-p-OMe-Phe-Pro-Lysψ{COCO}-OIP (111 mg) from N-(t-butyloxycarbonylmethyl)-N-Boc-D-p-OMe-Phe-Pro-Lys(Boc)ψ[CHOHCO]-OIP (235 mg).

Rₜ(LC): 26.22 min, A 20 %, B 80 %, C 0 % to A 20 %, B 20 %, C 60 % in 40 min.
EXAMPLE 33

HOOC-CH₂-D-Cha-Pro-Lys⁴[COO⁻]-NH-Benzyl

(a) N-(t-BuIoxy carbonylmethyl)-N-Boc-D-Cha-Pro-Lys(Boc)⁴[CHOHCO]-OH

N-(t-BuIoxy carbonylmethyl)-N-Boc-D-Cha-Pro-Lys(Boc)⁴[CHOHCO]-OEt (2 g), prepared as described in example 3, was dissolved in 200 ml of a dioxane/water mixture 9/1 v/v. Next, the pH was adjusted to 12 with 1N NaOH and the solution was stirred at room temperature for 6h. After that the solution was cooled on an icebath (4 ºC) and the pH was lowered to 2 with 1N HCl. The resulting solution was extracted three times with dichloromethane, dried on sodium sulfate and evaporated to dryness. Yield was 2 g. TLC: Rₜ=0.08, silica gel, dichloromethane/methanol 9/1 v/v.

(b) N-(t-BuIoxy carbonylmethyl)-N-Boc-D-Cha-Pro-Lys(Boc)⁴[CHOHCO]-NH-Benzyl

N-(t-BuIoxy carbonylmethyl)-N-Boc-D-Cha-Pro-Lys(Boc)⁴[CHOHCO]-OH (400 mg) was dissolved in 10 ml N,N-dimethylformamide and 1-hydroxy-benzotriazole hydrate (110 mg), benzylamine (118 µl) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (124 mg) were added. The reaction mixture was stirred for 16 h at room temperature after which the mixture was poured in icecold 1.0 N HCl. Next, the waterlayer was extracted three times with ethyl acetate and the resulting organic layer was washed with water, 5% sodium hydrogencarbonate and water, dried on sodium sulfate and evaporated to dryness. Yield was 405 mg. TLC: Rₜ=0.8, silica gel, ethyl acetate/pyridine/acetic acid/water=655/31/18/7 v/v/v/v.

(c) N-(t-BuIoxy carbonylmethyl)-N-Boc-D-Cha-Pro-Lys(Boc)⁴[COO⁻]-NH-Benzyl

The Dess-Martin oxidation was performed as described in example 3 starting with 400 mg hydroxyamide, yielding 350 mg oxidized product TLC: Rₜ=0.37, silica gel, dichloromethane/methanol 95/5 v/v.
(d) HOOC-CH$_2$-D-Cha-Pro-Lys$\Psi$(COCO)-NH-Benzy1

N-(t-Butyloxycarbonylmethyl)-N-Boc-D-Cha-Pro-Lys(Boc)$\Psi$(COCO)-NH-Benzyl (350 mg) was deprotected as described in example 3.

The lyophilized crude product (338 mg of di trifluoro acetate salt) was charged onto a preparative HPLC DeltaPak RP-C$_{18}$ using a gradient elution system of 20% A/80% B to 20% A/50% B/45% C over 45 min at a flow rate of 80 ml. Yield: 165 mg of HOOC-CH$_2$-D-Cha-Pro-Lys$\Psi$(COCO)-NH-Benzy1.

Rt(LC): 36.17 min, A 20 %, B 80 %, C 0 % to A 20 %, B 20 %, C 60 % in 40 min.

EXAMPLE 34

HOOC-CH$_2$-D-Cha-Pro-Lys$\Psi$(COCO)-NH$_2$

The coupling (119 µl N-methylmorpholine was added), oxidation, deprotection and purification were done according to procedures described in example 33, starting from N-(t-Butyloxycarbonylmethyl)-N-Boc-D-Cha-Pro-Lys(Boc)$\Psi$(CHOHCO)-OH (400 mg). After HPLC purification the yield was 119 mg.

Rt(LC): 21.97 min, A 20 %, B 80 %, C 0 % to A 20 %, B 20 %, C 60 % in 40 min.

EXAMPLE 35

HOOC-CH$_2$-D-Cha-Pro-Lys$\Psi$(COCO)-NH-Me

The coupling, oxidation, deprotection and purification were done according to procedures described in example 33, starting from N-(t-Butyloxycarbonylmethyl)-N-Boc-D-Cha-Pro-Lys(Boc)$\Psi$(CHOHCO)-OH (400 mg). After HPLC purification the yield was 185 mg.

Rt(LC): 25.22 min, A 20 %, B 80 %, C 0 % to A 20 %, B 20 %, C 60 % in 40 min.
EXAMPLE 36

**HOOC-CH$_2$-D-Cha-Pro-Lys[COCO]-Azetidine**

The coupling, oxidation, deprotection and purification were done according to procedures described in example 33, starting from N-(t-Butoxy carbonylmethyl)-N-Boc-D-Cha-Pro-Lys(Boc)[CHOHCO]-OH (400 mg). After HPLC purification the yield was 85 mg.

Rt(LC): 29.11 min, A 20 %, B 80 %, C 0 % to A 20 %, B 20 %, C 60 % in 40 min.

EXAMPLE 37

**HOOC-CH$_2$-D-Cha-Pro-Lys[COCO]-NH-CH$_2$-Benzyl**

The coupling, oxidation, deprotection and purification were done according to procedures described in example 33, starting from N-(t-Butoxy carbonylmethyl)-N-Boc-D-Cha-Pro-Lys(Boc)[CHOHCO]-OH (400 mg). After HPLC purification the yield was 151 mg.

Rt(LC): 38.61 min, A 20 %, B 80 %, C 0 % to A 20 %, B 20 %, C 60 % in 40 min.

EXAMPLE 38

**HOOC-CH$_2$-D-p-OMe-Phe-Pro-Lysw[COCO]-Azetidine**

(a) Cbz-Lys(Boc)[CHOHCO]-OH

Cbz-Lys(Boc)[CHOHCO]-OMe (2.98 g) was dissolved in dioxane/water=7/3 v/v (40 ml) and treated with 1M sodium hydroxide solution (4.5 ml) portionwise over 4 h. at room temperature, keeping the pH at 10-10.5. The reaction mixture was diluted with water, 2M hydrogen chloride solution was added until pH 2.0 and the water layer was extracted with dichloromethane. The combined organic phases were washed with water, brine and dried over sodium sulfate, filtered and concentrated in vacuo to yield Cbz-Lys(Boc)[CHOHCO]-OH (2.70 g).
TLC: \( R_f = 0.53 \), silica gel, ethyl acetate/pyridine/acetic acid/water = 232/31/18/7 v/v/v/v.

(b) \text{Cbz-Lys(Boc)Ψ[CHOHCO]-Azetidin}

1-Hydroxy-benzotriazole hydrate (1.4 g), azetidine hydrochloride (1.2 g), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (1.4 g) and N-methylmorpholine (1.4 ml) were added to a solution of Cbz-Lys(Boc)Ψ[CHOHCO]-Azetidin (2.7 g) in N,N-dimethylformamide (35 ml). The reaction mixture was stirred for 16 h at room temperature. The mixture was poured into water and extracted with ethyl acetate. The combined organic layers were washed with 0.1N hydrochloric acid solution, water, 5% sodium hydrogen carbonate solution and brine. The organic layer was dried over sodium sulfate, filtered and evaporated. The residuum was purified by chromatography on silica gel (elucent: gradient of heptane/ethyl acetate 1/2 v/v to ethyl acetate/methanol 9/1 v/v) to yield Cbz-Lys(Boc)Ψ[CHOHCO]-Azetidin (2.26 g).

TLC: \( R_f = 0.65 \), silica gel, ethyl acetate/pyridine/acetic acid/water = 376/31/18/7 v/v/v/v.

(c) \text{H-Lys(Boc)Ψ[CHOHCO]-Azetidin}

To a solution of Cbz-Lys(Boc)Ψ[CHOHCO]-Azetidin (184 mg) in methanol (6 ml) were added 10% palladium on activated carbon (60 mg) and 2N HCl (0.2 ml) and this suspension was hydrogenated at atmospheric pressure for 2 hours at room temperature. The palladium catalyst was removed by filtration and the filtrate was concentrated in vacuo to yield H-Lys(Boc)Ψ[CHOHCO]-Azetidin HCl (180 mg, crude).

TLC: \( R_f = 0 \), silica gel, ethyl acetate/pyridine/acetic acid/water = 520/31/18/7 v/v/v/v.

(d) \text{N-(t-butyloxy carbonylmethyl)-N-Boc-D-p-OMe-Phe-Pro-Lys(Boc)Ψ[COCO]-Azetidin}

N-(t-Butyloxy carbonylmethyl)-N-Boc-D-p-OMe-Phe-Pro-OH (208 mg) was coupled with H-Lys(Boc)Ψ[CHOHCO]-Azetidin according to the procedures described in example 1 to give N-(t-butyloxy carbonylmethyl)-N-Boc-D-p-OMe-Phe-Pro-Lys(Boc)Ψ[CHOHCO]-Azetidin (259 mg).
N-(t-Butyloxy carbonylmethyl)-N-Boc-D-p-OMe-Phe-Pro-Lys(Boc)Ψ[CHOHCO]-Azetidine was used in the Dess Martin oxidation as described in example 3 to yield the title compound (246 mg).

TLC: \( R_f = 0.33 \), silica gel, dichloromethane/methanol = 95/5 v/v.

(e) HOOC-CH₂-D-p-OMe-Phe-Pro-LysΨ[COCO]-Azetidine

N-(t-Butyloxy carbonylmethyl)-N-Boc-D-p-OMe-Phe-Pro-Lys(Boc)Ψ[CHOHCO]-Azetidine (246 mg, crude) was deprotected and purified using the procedure described in example 29 to give HOOC-CH₂-D-p-OMe-Phe-Pro-LysΨ[COCO]-Azetidine (151 mg).

\( R_t(LC) : 24.46 \text{ min, A } 20 \% , \text{ B } 80 \% , \text{ C } 0 \% \text{ to A } 20 \% , \text{ B } 20 \% , \text{ C } 60 \% \text{ in 40 min.} \)

EXAMPLE 39

HOOC-CH₂-D-p-OMe-Phe-Azt-LysΨ[COCO]-Azetidine

The procedure used to prepare HOOC-CH₂-D-p-OMe-Phe-Pro-LysΨ[COCO]-Azetidine from N-(t-butyloxy carbonylmethyl)-N-Boc-D-p-OMe-Phe-Pro-Lys(Boc)Ψ[CHOHCO]-Azetidine (example 38) was also used to prepare HOOC-CH₂-D-p-OMe-Phe-Azt-LysΨ[COCO]-Azetidine. \( 183 \text{ mg} \) from N-(t-butyloxy carbonylmethyl)-N-Boc-D-p-OMe-Phe-Azt-Lys(Boc)Ψ[CHOHCO]-Azetidine \( 284 \text{ mg} \).

\( R_t(LC) : 21.8 \text{ min, } 20\% \text{ A/80\% B to 20\% A/20\% B/60\% C in 40 min.} \)

EXAMPLE 40

HOOC-CH₂-D-p-OMe-Phe-Pro-LysΨ[COCO]-NH₂

The procedure used to prepare HOOC-CH₂-D-Cha-Pro-LysΨ[COCO]-NH₂ from N-(t-butyloxy carbonylmethyl)-N-Boc-D-Cha-Pro-Lys(Boc)Ψ[CHOHCO]-NH₂ (example 34) was
also used to prepare HOOC-CH$_2$-D-p-OMe-Phe-Pro-Lys[COCO]-NH$_2$ (192 mg) from N-(t-butyloxy carbonylmethyl)-N-Boc-D-p-OMe-Phe-Pro-Lys(Boc)$\Psi$[CHOHCO]-NH$_2$ (422 mg). 

$R_f$(LC) : 18.0 min.; 20% A/80% B to 20% A/20% B/60% C in 40 min.

EXAMPLE 41

**HOOC-CH$_2$-D-Cha-Azt-Lys$\Psi$[COCO]-Azetidine**

(a) \textit{N-}(t-Butyloxy carbonylmethyl)-N-Boc-D-Cha-Azt-Lys(Boc)$\Psi$[CHOHCO]-Azetidine

N-(t-Butyloxy carbonylmethyl)-N-Boc-D-Cha-Azt-OH (prepared according to procedure no 21) was coupled to H-Lys(Boc)$\Psi$[CHOHCO]-Azetidine (prepared according to procedure no 38) in a similar manner as described in example 1. After flash chromatography on silica using ethyl acetate/heptane 1/1 v/v the yield, starting from 375 mg of the dipeptide, was 540 mg. 

TLC: $R_f=0.90$, silica gel, ethyl acetate/pyridine/acetic acid/water=63/20/6/11 v/v/v/v.

(b) \textit{N-}(t-Butyloxy carbonylmethyl)-N-Boc-D-Cha-Azt-Lys(Boc)$\Psi$[COCO]-Azetidine

N-(t-Butyloxy carbonylmethyl)-N-Boc-D-Cha-Azt-Lys(Boc)$\Psi$[CHOHCO]-Azetidine (540 mg) was oxidized using Dess-Martin reagent according to procedures described in example 1 with a reaction time of 64 h at room temperature. Yield is 580 mg (crude). 

TLC: $R_f=0.66$, silica gel, ethyl acetate/pyridine/acetic acid/water=163/20/6/11 v/v/v/v.

(c) \textit{HOOC-CH$_2$-D-Cha-Azt-Lys$\Psi$[COCO]-Azetidine}

N-(t-Butyloxy carbonylmethyl)-N-Boc-D-Cha-Azt-Lys(Boc)$\Psi$[COCO]-Azetidine (580 mg) was dissolved in 30 ml of 3 N HCl in dioxane, followed after 4.5 h at room temperature and after evaporation to dryness, by dissolving the residu in 10 ml of trifluoroacetic acid/water 9/1 v/v for 2 h at room temperature.

Purification was done according to the procedure described in example 1. Yield is 151 mg. 

$R_f$(LC) : 25.0 min.; 20% A/80% B to 20% A/20% B/60% C in 40 min.
EXAMPLE 42

HOOC-CH$_2$-D-Coa-Pro-Lys$_3$-[COCO]-Azetidine

N-(t-Butyloxycarbonylmethyl)-N-Boc-D-Coa-Pro-OH (prepared according to procedure no 11) was coupled to H-Lys(Boc)$_3$-[CHOHCO]-Azetidine (prepared according to procedure no 38) in a similar manner as described in example 1. Subsequently, oxidation (5 h), deprotection and purification were done in analogous manner as described in example 1. Yield is 262 mg. Rt(LC): 35.93 min, A 20 %, B 80 %, C 0 % to A 20 %, B 20 %, C 60 % in 40 min.

EXAMPLE 43

HOOC-CH$_2$-D-Dpa-Pro-Lys$_3$-[COCO]-Azetidine

N-(t-Butyloxycarbonylmethyl)-N-Boc-D-Dpa-Pro-OH (prepared according to procedure no 9) was coupled to H-Lys(Boc)$_3$-[CHOHCO]-Azetidine (prepared according to procedure no 38) in a similar manner as described in example 1. Subsequently, oxidation (24 h), deprotection and purification were done in analogous manner as described in example 1. Yield is 287 mg. Rt(LC): 33.97 min, A 20 %, B 80 %, C 0 % to A 20 %, B 20 %, C 60 % in 40 min.

EXAMPLE 44

HOOC-CH$_2$-D-p-Cl-Phe-Pro-Lys$_3$-[COCO]-Azetidine

(a) N-(t-Butyloxycarbonylmethyl)-N-Boc-D-p-Cl-Phe-Pro-Lys(Boc)$_3$-[CHOHCO]-Azetidine

N-(t-Butyloxycarbonylmethyl)-N-Boc-D-p-Cl-Phe-Pro-OH (see example 10) was coupled with H-Lys(Boc)$_3$-[CHOHCO]-Azetidine (see example 38) using procedures described in example 1 to give N-(t-butyloxycarbonylmethyl)-N-Boc-D-p-Cl-Phe-Pro-Lys(Boc)$_3$-[CHOHCO]-Azetidine.

TLC: Rf= 0.7, silica gel, ethyl acetate/pyridine/acetic acid/water 376/31/18/7 v/v/v/v.
(b) \(N-(t\)-Butyloxycarbonylmethyl\)-N-Boc-D-p-Cl-Phe-Pro-Lys(Boc)\(\Psi[\text{COCO}]\)-Azetidine

\(N-(t\)-Butyloxycarbonylmethyl\)-N-Boc-D-p-Cl-Phe-Pro-Lys(Boc)\(\Psi[\text{CHOHCO}]\)-Azetidine was oxidised using the Dess Martin reagent according the procedure described example 1.

TLC: Rf = 0.85, silica gel, ethyl acetate/pyridine/acetic acid/water 520/31/18/7 v/v/v/v.

(c) \(\text{HOOC-CH}_2\text{-D-p-Cl-Phe-Pro-Lys}[\text{COCO}]\)Azetidine

The title compound was obtained by removal of the protective groups and subsequent purification according the procedure described in example 1. Yield is 125 mg.

Rt(LC): 30.3 min, A 20 %, B 80 %, C 0 % to A 20 %, B 20 %, C 60 % in 40 min.

**EXAMPLE 45**

\(\text{HOOC-CH}_2\text{-D-m-Cl-Phe-Pro-Lys}[\text{COCO}]\)Azetidine

(a) \(N-(t\)-Butyloxycarbonylmethyl\)-N-Boc-D-m-Cl-Phe-Pro-Lys(Boc)\(\Psi[\text{CHOHCO}]\)-Azetidine

\(N-(t\)-Butyloxycarbonylmethyl\)-N-Boc-D-m-Cl-Phe-Pro-OH (see example 8) was coupled with H-Lys(Boc)\(\Psi[\text{CHOHCO}]\)-Azetidine (see example 38) using procedures described in example 1 to give \(N-(t\)-butyloxycarbonylmethyl\)-N-Boc-D-p-Cl-Phe-Pro-Lys(Boc)\(\Psi[\text{CHOHCO}]\)-Azetidine

TLC: Rf = 0.76, silica gel, ethyl acetate/pyridine/acetic acid/water 376/31/18/7 v/v/v/v.

(b) \(N-(t\)-Butyloxycarbonylmethyl\)-N-Boc-D-m-Cl-Phe-Pro-Lys(Boc)\(\Psi[\text{COCO}]\)-Azetidine

\(N-(t\)-Butyloxycarbonylmethyl\)-N-Boc-D-m-Cl-Phe-Pro-Lys(Boc)\(\Psi[\text{CHOHCO}]\)-Azetidine was oxidised using the Dess Martin reagent according the procedure described example 1.

TLC: Rf = 0.44, silica gel, dichloromethane/methanol 95/5 v/v.
(c) HOOC-CH$_2$-D-m-Cl-Phe-Pro-Lys$_3$(COCO)Azetidine

The title compound was obtained by removal of the protective groups and subsequent purification according the procedure described in example 1. Yield is 272 mg.

Rt(LC): 28.50 min, A 20 %, B 80 %, C 0 % to A 20 %, B 20 %, C 60 % in 40 min.

EXAMPLE 46

HOOC-CH$_2$-CH$_2$-D-Cha-Pro-Lys(2-thiazoyl)

(a) N-(2-(t-Butyloxycarbonyl)ethyl)-D-Cha-Pro-Lys(Cbz)-(2-thiazoyl)

Boc-D-Cha-Pro-Lys(Cbz)-(2-thiazoyl) (256 mg) was dissolved in dichloromethane (1.5 mL) and trifluoroacetic acid (1.5 mL) and stirred for 1 hour at room temperature. The reaction mixture was concentrated in vacuo and coevaporated with toluene. The residue was dissolved in acetonitrile (2 mL), adjusted to pH 7 using N,N-diisopropylethylamine and tert-butyl acrylate (3 mL) was added. After stirring at room temperature for 17 days maintaining the pH at 7 using N,N-diisopropylethylamine the reaction mixture was concentrated under reduced pressure. The residue was dissolved in ethyl acetate, washed with water, dried over sodium sulphate and concentrated. The residue was purified using chromatography (silica gel, gradient ethyl acetate/toluene = 1/1 v/v to ethyl acetate) to yield N-(2-(t-butyloxycarbonyl)ethyl)-D-Cha-Pro-Lys(Cbz)-(2-thiazoyl)(125 mg).

TLC: R$_f$ = 0.7, silica gel, ethyl acetate

(b) HOOC-CH$_2$-CH$_2$-D-Cha-Pro-Lys(2-thiazoyl)

The procedure used to prepare (HOOC-CH$_2$)$_2$-D-Cha-Pro-Lys-(2-thiazoyl) from N-(di-t-butyloxycarbonylmethyl)-D-Cha-Pro-Lys(Cbz)-(2-thiazoyl) (example 4) was used. N-(2-(t-Butyloxycarbonyl)ethyl)-D-Cha-Pro-Lys(Cbz)-(2-thiazoyl)(125 mg) yielded HOOC-CH$_2$-CH$_2$-D-Cha-Pro-Lys-(2-thiazoyl) as two diastereomers that could be separated.
A: Yield 40 mg Rt(LC): 30.2 min, A 20 %, B 80 %, C 0 % to A 20 %, B 20 %, C 60 % in 40 min.

B: Yield 37 mg Rt(LC): 32.8 min, A 20 %, B 80 %, C 0 % to A 20 %, B 20 %, C 60 % in 40 min.

EXAMPLE 47

\[(\text{HOOC-CH}_2)(\text{CH}_3)\text{-D-Cha-Pro-Lys-(2-thiazolyl)}\]

(a) \(N-(\text{t-Butyloxycarbonylmethyl})-N-(\text{methyl})\text{-D-Cha-Pro-Lys(Cbz)-(2-thiazolyl)}\)

Boc-D-Cha-Pro-Lys(Cbz)-(2-thiazolyl) (0.55 g) was dissolved in dichloromethane (2.5 ml) and trifluoroacetic acid (2.5 ml) and stirred for 1 hour at room temperature. The reaction mixture was concentrated in vacuo and coevaporated with toluene. The residue was dissolved in acetonitrile (10 ml), \(N,N\)-diisopropylethylamine (0.18 ml) and tert-butyl bromoacetate. After stirring the reaction mixture at room temperature for 16 hours maintaining the pH at 7 using \(N,N\)-diisopropylethylamine iodomethane (0.1 ml) was added and the reaction mixture was heated at 34 °C keeping the pH at 7.5 using \(N,N\)-diisopropylethylamine. After 5 hours the reaction mixture was concentrated in vacuo. The residue was dissolved in ethyl acetate, washed with water, dried over sodium sulphate and concentrated. The residue was purified using chromatography (silica gel, ethyl acetate/toluene = 1/1 v/v) to yield \(N-(\text{t-butyloxycarbonylmethyl})-N-(\text{methyl})\text{-D-Cha-Pro-Lys(Cbz)-(2-thiazolyl)}\) (173 mg).

TLC: \(R_f = 0.3\), silica gel, ethyl acetate/toluene = 1/1 v/v

(b) \(\text{(HOOC-CH}_2)(\text{CH}_3)\text{-D-Cha-Pro-Lys-(2-thiazolyl)}\)

\(N-(\text{t-Butyloxycarbonylmethyl})-N-(\text{methyl})\text{-D-Cha-Pro-Lys(Cbz)-(2-thiazolyl)}\) (0.32 g) was treated with trifluoroacetic acid/thioanisole = 10/1 (v/v) (4.4 ml) and stirred for 4 hours at room temperature. The reaction mixture was concentrated in vacuo, coevaporated with toluene and tritutrated with diethylether. The residue was dissolved in a small amount of dichloromethane (2 ml) and this solution was added to diethylether (100 ml). The precipitate was isolated,
dissolved in water and charged onto a preparative HPLC DeltaPak RP-C18 using a gradient elution system of 20% A/80% B/0% C to 20% A/40% B/40% C over 45 min at a flow rate of 50 mL. Yield: 94 mg of (HOOC-CH₂)(CH₃)-D-Cha-Pro-Lys-(2-thiazolyl).
Rt(LC): 33.2 min, A: 20%, B: 80%, C: 0% to A: 20%, B: 20%, C: 60% in 40 min.

EXAMPLE 48

(HOOC-CH₂)(Phenylmethyl)-D-Cha-Pro-Lys-(2-thiazolyl)

(a) N-(t-Butyloxy carbonylmethyl)-N-(phenylmethyl)-D-Cha-Pro-Lys(Cbz)-(2-thiazolyl)

t-Butyl-bromoacetate (0.28 ml) was added to a stirred solution H-D-Cha-Pro-Lys(Cbz)-(2-thiazolyl). TFA (710 mg) (see example 4) in 10 ml of acetonitrile. The pH of the mixture was adjusted to 8 with diisopropylethylamine and the mixture was stirred for 4 days at room temperature. Phenylmethylbromide (0.17 ml) was subsequently added, maintaining the pH at 8.5 by addition of diisopropylethylamine, and the reaction mixture was stirred for an additional 48 hours at room temperature, followed by evaporation in vacuo. The residue was dissolved in ethyl acetate and the solution was washed with water and brine, dried on sodium sulphate and evaporated in vacuo. Chromatography over silica gel in toluene/ethyl acetate 7/3 v/v gave 360 mg of N-(t-butyloxy carbonylmethyl)-N-(phenylmethyl)-D-Cha-Pro-Lys(Cbz)-(2-thiazolyl).

TLC : Rₜ = 0.80, silica gel, ethyl acetate/pyridine/acetic acid/water 232/31/18/7 v/v/v/v.

(b) (HOOC-CH₂)(Phenylmethyl)-D-Cha-Pro-Lys-(2-thiazolyl)

The title compound was obtained by removal of the protective groups and subsequent purification according the procedure described in example 5. Yield is 115 mg
Rt(LC): 45.2 min, A 20 %, B 80 %, C 0 % to A 20 %, B 20 %, C 60 % in 40 min.

EXAMPLE 49

(HOOC-CH₂)(2-Propenyl)-D-Cha-Pro-Lys-(2-thiazolyl)
(a) N-(t-Butyloxycarbonylmethyl)-N-(2-propenyl)-D-Cha-Pro-Lys(Cbz)-(2-thiazoyl)

t-Butyl-bromoacetate (0.55 ml) was added to a stirred solution H-D-Cha-Pro-Lys(Cbz)-(2-thiazoyl) TFA (1.55 g) (see example 4) in 25 ml of acetonitrile. The pH of the mixture was adjusted to 8 with diisopropylethylamine and the mixture was stirred for 2 days at room temperature. Allyl chloride (0.23 ml) was subsequently added, maintaining the pH at 8.5 by addition of diisopropylethylamine, and the reaction mixture was stirred for an additional 48 hours at room temperature, followed by evaporation in vacuo. The residue was dissolved in ethyl acetate and the solution was washed with water and brine, dried on sodium sulphate and evaporated in vacuo. Chromatography over silica gel in toluene/ethyl acetate 7/3 v/v gave 650 mg of N-(t-butyloxycarbonylmethyl)-N-(2-propenyl)-D-Cha-Pro-Lys(Cbz)-(2-thiazoyl).
TLC : Rf = 0.85, silica gel, toluene/ethyl acetate 1/1 v/v.

(b) (HOOC-CH2)(2-Propenyl)-D-Cha-Pro-Lys-(2-thiazoly)

The title compound was obtained by removal of the protective groups and subsequent purification according the procedure described in example 5. Yield is 250 mg.
Rt(LC): 35.9 min, A 20 %, B 80 %, C 0 % to A 20 %, B 20 %, C 60 % in 40 min.

EXAMPLE 50

BzOOC-CH2-SO2-D-Cha-Pro-Lys-(2-thiazoly)

(a) N-Benzylxocarbonylmethylsulfonyl-D-Cha-Pro-Lys(Cbz)-(2-thiazoly)

Boc-D-Cha-Pro-Lys(Cbz)-(2-thiazoly) (1.05 g) was dissolved in 3 M hydrogen chloride in dioxane (5 ml) and stirred for 40 minutes at room temperature. The reaction mixture was concentrated in vacuo and coevaporated with toluene. The residue was dissolved in dichloromethane (10 ml) and under a nitrogen atmosphere cooled in an ice bath. With stirring benzylxocarbonylmethylsulfonyle chloride (crude product obtained from 0.20 ml chlorosulfonylacetylchloride as described by S. Vanwetswinkel, J. Fastrez and J. Marchand-Brynaert in J.
Antibiotics 47, 1041 (1994)) dissolved in dichloromethane (5ml) was added followed by N,N-diisopropylethylamine (0.70 ml) in small portions. The ice bath was removed and the reaction mixture was stirred at room temperature for an additional 30 minutes. The mixture was washed with water, dried over sodium sulphate and evaporated to dryness in vacuo. The residue was purified using chromatography (silica gel, ethyl acetate/heptanes = 1/1 v/v) to yield N-benzyloxycarbonylmethylsulfonyl-D-Cha-Pro-Lys(Cbz)-(2-thiazolyl) (0.745 g).

TLC: Rₜ = 0.4, silica gel, ethyl acetate/heptanes = 2/1 v/v

(b) BzlOOC-CH₂-SO₂-D-Cha-Pro-Lys-(2-thiazolyl)

The procedure used to prepare (HOOC-CH₂)(CH₃)-D-Cha-Pro-Lys-(2-thiazolyl) from N-(t-butyloxycarbonylmethyl)-N-(methyl)-D-Cha-Pro-Lys(Cbz)-(2-thiazolyl) (example 47) was also used to prepare BzlOOC-CH₂-SO₂-D-Cha-Pro-Lys-(2-thiazolyl) (94 mg) from N-benzyloxycarbonylmethylsulfonyl-D-Cha-Pro-Lys(Cbz)-(2-thiazolyl) (301 mg).

Rₜ(LC): 34.0 min, A: 20%, B: 60%, C: 20% to A: 20%, B: 0%, C: 80% in 40 min.

EXAMPLE 51

HOOC-CH₂-SO₂-D-Cha-Pro-Lys-(2-thiazolyl)

N-benzyloxycarbonylmethylsulfonyl-D-Cha-Pro-Lys(Cbz)-(2-thiazolyl) (46 g) was cooled in an ice bath and treated with trifluoroacetic acid/thioanisole/bromotrimethylsilane = 50/6/6.6 (v/v/v) (12 ml). After stirring for 4 hours, the reaction mixture was concentrated in vacuo, coevaporated with toluene and triturated with diethylether. The residue was dissolved in a small amount of dichloromethane (2 ml) and this solution was added to diethylether (100 ml). The precipitate was isolated, dissolved in water and charged onto a preparative HPLC DeltaPak RP-C₁₈ using a gradient elution system of 20% A/80% B/0% C to 20% A/30% B/50% C over 45 min at a flow rate of 50 ml/min. Yield: 213 mg of HOOC-CH₂SO₂-D-Cha-Pro-Lys-(2-thiazolyl).

Rₜ(LC): 25.1 min, A: 20%, B: 80%, C: 0% to A: 20%, B: 20%, C: 60% in 40 min.
EXAMPLE 52

(EtOOC-CH$_2$)(Me)-D-Cha-Pro-Lys-(2-thiazolyl)

N-(t-Butyloxy carbonylmethyl)-N-Boc-N-Me-D-Cha-Pro-Lys(Cbz)-(2-thiazolyl) was prepared according a similar manner as described in example 47. The protected tripeptide was dissolved in 3M HCl/ethanol and stirred for 24 at room temperature. After evaporation the residue was dissolved in trifluoroacetic acid/thioanisole=10/1 v/v and stirred for 4 h at room temperature. After evaporation of the solvent, the product was purified with HPLC as described in example 1. Yield: 38 mg. 

R$_s$(LC): 38.40 min.; 20% a/80% B to 20% A/20% B/60% C in 40 min.

EXAMPLE 53

HOOC-CH$_2$-D-p-OMe-Cha-Pro-Lys$_w$(COCO)-OH

(a). N-(t-Butyloxy carbonylmethyl)-N-Boc-D-p-OMe-Cha-OH

A stirred suspension of N-(t-Butyloxy carbonylmethyl)-N-Boc-D-p-OMe-Phe-OH (0.97 g) and platinum(IV)oxide (0.39 g) in ethanol (100 ml) was hydrogenated at atmospheric pressure at room temperature for 12 days. The platinum catalyst was removed by filtration and the solvent was removed by evaporation at reduced pressure. The residue was purified using chromatography (silica gel, ethyl acetate/heptanes = 1/1 v/v) to yield 151 mg of the title compound.

TLC: R$_f$ = 0.2, silica gel, ethyl acetate/heptanes = 1/1 v/v

(b). HOOC-CH$_2$-D-p-OMe-Cha-Pro-Lys$_w$(COCO)-OH

N-(t-Butyloxy carbonylmethyl)-N-Boc-D-p-OMe-Cha-OH (0.15 g) was coupled with H-Pro-OBzl.HCl, hydrogenated, coupled with H-Lys(Boc)$_\Psi$(CHOHCO)-OMe.HCl, saponified,
oxidized using the Dess Martin reagent and the protecting groups were removed using trifluoroacetic acid as described in example 1 to afford the title compound (46 mg).

R<sub>4</sub>(LC) : 17.2 min.; 20% A/80% B to 20% A/20% B/60% C in 40 min.

EXAMPLE 54

(HOOC-CH<sub>2</sub>)(Me)-D-Cha-Pro-Lys<sup>Ψ</sup>[COCO]-OH

N-((t-Butyloxycarbonylmethyl)-N-Me-D-Cha-Pro-Lys(Boc)<sup>Ψ</sup>[CHOHCO]-OCH<sub>3</sub> (100 mg) was prepared according a similar manner as described in example 1. The hydroxyester was oxidized according to the following procedure; A solution of 130 µl oxalychloride in dichloromethane (5 ml) at -40 °C was treated with 212 µl DMSO and stirred for 15 min. The hydroxyester was added and the resulting solution was stirred at -40 °C for 1 h. Triethylamine (1 ml) was added and the mixture was allowed to warm to ambient temperature. After 3 h stirring at room temperature, 5% sodium thiosulfate solution was added and the mixture was stirred for 30 min at room temperature. The organic layer was separated, washed with water and brine, dried on sodium sulphate and evaporated. The crude ketoester was saponified and deprotected as described in example 1. The product was purified with HPLC as described in example 1.

Yield : 21 mg.

R<sub>4</sub>(LC) : 23.7 min.; 20% A/80% B to 20% A/20% B/60% C in 40 min.

EXAMPLE 55

HOOC-CH<sub>2</sub>-D-Cha-Pro-Arg<sup>Ψ</sup>[COCO]-OH

(a) H-Arg(Boc)[CHOHCO]-OMe HCl

To a solution of 3-[4-(1,1-dimethylethoxycarbonylamino)cyclohexyl]-2-hydroxy-3-nitropropionic acid methyl ester (Lyle et al, Bioorg. Med. Chem. Lett., 7, 67-72 (1997)) (294 mg) in methanol (100 mL) was added 2N hydrochloric acid (0.425 mL) and 10 % palladium on activated carbon powder (0.45 g) and this suspension was hydrogenated at atmospheric
pressure at room temperature for 16 hours. The palladium catalyst was removed by filtration and the solvent was removed by evaporation at reduced pressure yielding H-Acg(Boc)Ψ[CHOHCO]-OMe.HCl (289 mg) as a mixture of diastereomers. 

TLC: Rf= 0.26, silica gel, ethyl acetate/pyridine/acetic acid/water=232/31/18/7 v/v/v/v.

(b) HOOC-CH₂-D-Cha-Pro-AcgΨ[COCO]-OH

The experimental procedures described in example 1 were used to prepare the title compound. N-(t-Butyloxycarbonylmethyl)-N-Boc-D-Cha-Pro-OH (0.44 g) was coupled with H-Acg (Boc)Ψ[CHOHCO]-OMe.HCl (0.31 g) to give N-(t-butyloxycarbonylmethyl)-N-Boc-D-Cha-Pro-Acg(Boc)Ψ[CHOHCO]-OMe (0.51 g). This peptide (0.48 g) was saponified, oxidized using the Dess Martin reagent, the protecting groups were removed using trifluoroacetic acid and purified using HPLC to afford of HOOC-CH₂-D-Cha-Pro-AcgΨ[COCO]-OH (0.20 g) as a mixture of diastereomers

Rf(LC) : 23.0 min. and 23.2 min.; 20% A/80% B to 20% A/20% B/60% C in 40 min.

EXAMPLE 56

(HOOC-CH₃)(Me)-D-Cha-Pro-Lys-(2-benzimidazolyl)

Saponification of Cbz-Lys(Boc)Ψ[CHOHCO]-OMe and coupling, according to the procedures of example 33, of the resulting acid with mono-N-Boc-phenylenediamine yielded Cbz-Lys(Boc)Ψ[CHOHCO]-NH-[ortho(BocNH)phenyl] (TLC: Rf= 0.92, silica gel, ethyl acetate/pyridine/acetic acid/water=63/20/6/11 v/v/v/v). This product is cyclized (according to Tamura et al., Bioorg. Med. Chem. Lett, 7, 1359 (1997)), protected with the Boc group, and oxidized to produce Cbz-Lys(Boc)-(2-benzimidazolyl). The procedures described in example 1 and 4 are used to prepare the title compound from Cbz-Lys(Boc)-(2-benzimidazolyl).

EXAMPLE 57

The following compounds are prepared using the methods of the present invention:
- HOOC-CH$_2$-D-Leu-Pro-Lys-COOH
- HOOC-CH$_2$-D-Tyr-Pro-Lys-COOH
- HOOC-CH$_2$-D-p-F-Phe-Pro-Lys-COOH
- HOOC-CH$_2$-D-p-CF$_3$-Phe-Pro-Lys-COOH
- HOOC-CH$_2$-D-m-F-Phe-Pro-Lys-COOH
- HOOC-CH$_2$-D-m-Br-Phe-Pro-Lys-COOH
- HOOC-CH$_2$-D-m-OCH$_3$-Phe-Pro-Lys-COOH
- HOOC-CH$_2$-D-m-CF$_3$-Phe-Pro-Lys-COOH
- HOOC-CH$_2$-D-m-OH-Phe-Pro-Lys-COOH
- HOOC-CH$_2$-D-o-F-Phe-Pro-Lys-COOH
- HOOC-CH$_2$-D-o-Cl-Phe-Pro-Lys-COOH
- HOOC-CH$_2$-D-m,p-di-F-Phe-Pro-Lys-COOH
- HOOC-CH$_2$-D-o,p-di-Cl-Phe-Pro-Lys-COOH
- HOOC-CH(CH$_3$)$_2$-D-Cha-Pro-Lys-COOH
- (HOOC-CH$_2$)$_2$-D-Cha-Pro-Lys-(2-oxazolyl)
- (HOOC-CH$_2$)$_2$CH$_3$-D-Cha-Pro-Lys-(2-oxazolyl)
- HOOC-CH$_2$-D-Cha-3,3-Dmp-Lys-(2-thiazolyl)
- HOOC-CH$_2$-D-Cha-3,3-Dmp-Lys-(2-oxazolyl)
- HOOC-CH$_2$-D-Cha-Thz-Lys-COOH
- HOOC-CH$_2$-D-Cha-Thz-Lys-(2-thiazolyl)
- HOOC-CH$_2$-D-Cha-Ohi-Lys-(2-oxazolyl)
- HOOC-CH$_2$-D-Cha-Azt-Lys-(2-thiazolyl)
- HOOC-CH$_2$-D-Cha-Azt-Lys-(2-oxazolyl)
- HOOC-CH$_2$-D-Cha-Pec-Lys-(2-thiazolyl)
- HOOC-CH$_2$-D-Cha-Pec-Lys-(2-oxazolyl)
- HOOC-CH$_2$-D-Cha-Aza-Pro-Lys-COOH
- HOOC-CH$_2$-D-Cha-Aza-Pro-Lys-(2-thiazolyl)
- HOOC-CH$_2$-D-Cha-Aza-Pro-Lys-(2-oxazolyl)
- HOOC-CH$_2$-CH$_2$-D-p-OCH$_3$-Phe-Pro-Lys-COOH
- HOOC-CH$_2$-CH$_2$-D-Nle-Pro-Lys-COOH
- HOOC-CH₂-D-3,3-dicyclohexylalanine-Pro-Lys-COOH
- HOOC-CH₂-CH₂-D-3,3-dicyclohexylalanine-Pro-Lys-COOH
- HOOC-CH₂-CH₂-CH₂-D-Cha-Pro-Lys-COOH
- (N-BzlSO₂)-Asp-Pro-Lys-COOH
- (N-BzlSO₂)-Glu-Pro-Lys-COOH
- (N-BzlSO₂)-Aad-Pro-Lys-COOH
- (N-cyclooctyl)-Asp-Pro-Lys-COOH
- (N-cyclooctyl)-Glu-Pro-Lys-COOH
- (N-cyclooctyl)-Aad-Pro-Lys-COOH

EXAMPLE 58

Anti-thrombin assay

Thrombin (Factor IIa) is a factor in the coagulation cascade. The anti-thrombin activity of compounds of the present invention was assessed by measuring spectrophotometrically the rate of hydrolysis of the chromogenic substrate s-2238 exterted by thrombin. This assay for anti-thrombin activity in a buffer system was used to assess the IC₅₀-value of a test compound.

Test medium: Tromethamine-NaCl-polyethylene glycol 6000 (TNP) buffer. Reference compound: I2581 (Kabi) Vehicle: TNP buffer. Solubilisation can be assisted with dimethylsulphoxide, methanol, ethanol, acetonitrile or tert-butyl alcohol which are without adverse effects in concentrations up to 2.5 % in the final reaction mixture.

Technique

Reagents*: 1. Tromethamine-NaCl (TN) buffer. Composition of the buffer: Tromethamine (Tris) 6.057 g (50 mmol), NaCl 5.844 g (100 mmol), water to 1 l. The pH of the solution is adjusted to 7.4 at 37 °C with HCl (10 mmol/l⁻¹). 2. TNP buffer: Polyethylene glycol 6000 is dissolved in TN buffer to give a concentration of 3 g/l⁻¹. 3. S-2238 solution: One vial S-2238 (25 mg; Kabi Diagnostica, Sweden) is dissolved in 20 ml TN buffer to give a concentration of
1.25 mg·ml⁻¹ (2 mmol·l⁻¹). 4. Thrombin solution: Human thrombin (16 000 nKat·vial⁻¹; Centraal Laboratorium voor Bloedtransfusie, Amsterdam, The Netherlands) is dissolved in TNP buffer to give a stock solution of 835 nKat·ml⁻¹. Immediately before use this solution is diluted with TNP buffer to give a concentration of 3.34 nKat·ml⁻¹.

- *All ingredients used are of analytical grade
- For aqueous solutions ultrapure water (Milli-Q quality) is used.

Preparation of test and reference compound solutions

The test and reference compounds are dissolved in Milli-Q water to give stock concentrations of 10⁻² mol·l⁻¹. Each concentration is stepwise diluted with the vehicle to give concentrations of 10⁻³, 10⁻⁴ and 10⁻⁵ mol·l⁻¹. The dilutions, including the stock solution, are used in the assay (final concentrations in the reaction mixture: 3·10⁻³, 10⁻³, 3·10⁻⁴, 10⁻⁴, 3·10⁻⁵, 10⁻⁵, 3·10⁻⁶ and 10⁻⁶ mol·l⁻¹, respectively).

Procedure

At room temperature 0.075 ml and 0.025 ml test compound or reference compound solutions or vehicle are alternately pipetted into the wells of a microtiter plate and these solutions are diluted with 0.115 ml and 0.0165 ml TNP buffer, respectively. An aliquot of 0.030 ml S-2238 solution is added to each well and the plate is pre-heated and pre-incubated with shaking in an incubator (Amersham) for 10 min at 37 °C. Following pre-incubation the hydrolysis of S-2238 is started by addition of 0.030 ml thrombin solution to each well. The plate is incubated (with shaking for 30 s) at 37 °C. Starting after 1 min of incubation, the absorbance of each sample at 405 nm is measured every 2 min for a period of 90 min using a kinetic microtiter plate reader (Twinreader plus, Flow Laboratories).

All data are collected in an IBM personal computer using LOTUS-MEASURE. For each compound concentration (expressed in mol·l⁻¹ reaction mixture) and for the blank the absorbance is plotted versus the reaction time in min.

Evaluation of responses: For each final concentration the maximum absorbance was calculated from the assay plot. The IC₅₀-value (final concentration, expressed in µmol·l⁻¹, causing 50% inhibition of the maximum absorbance of the blank) was calculated using the logit
transformation analysis according to Hafner et al. (Arzneim.-Forsch./Drug Res. 1977; 27(II): 1871-3).

In the following table, $IC_{50}$-values of compounds of the invention are listed:

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<th>Example</th>
<th>$IC_{50}$-value ($\mu$M)</th>
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</table>
CLAIMS

1. A compound having the formula I

\[ R^a-\text{CH-C(O)-A-B-X} \]

wherein \( R^a \) is \(-\text{NR}^1 \text{R}^2 \) and \( R^b \) is \( R^3 \), or \( R^a \) is \( R^1 \) and \( R^b \) is \(-\text{NH-}(\text{SO}_2)_m \text{R}^3 \), \( m \) being 0 or 1;
wherein \( R^1 \) is \(-\text{(SO}_2)_n \text{alkylene-COOH} \) or an ester derivative thereof, \( n \) being 0 or 1;
and \( m \) and \( n \) are not 1 at the same time;
\( R^2 \) is selected from \( \text{H, (1-12C)alkyl, (2-12C)alkenyl, (3-8C)cycloalkyl,} \)
\( \text{(1-6C)alkylene(3-8C)cycloalkyl, (6-14C)aryl, (7-15C)aralkyl and -(1-6C)alkylene-COOH or} \)
an ester derivative thereof;
\( R^3 \) is a hydrophobic moiety;
or \( R^2 \) and \( R^3 \) are a 5- or 6-membered ring together with "N-C" to which they are bound,
which ring may be fused with an aliphatic or aromatic 6-membered ring;
A is an amino acid selected from proline, optionally containing a second heteroatom selected
from \( \text{N, O, or S, and optionally substituted with (1-6C)alkyl, (1-6C)alkoxy or halogen, 3,4-} \)
dehydropoline, 2-azetidine carboxylic acid, pipecolinic acid, octahyroindole-2-carboxylic
acid, 2-amino isobutyric acid or valine,
B is lysine or 4-aminocyclohexylglycine, and
\( X \) is \(-\text{CHF}_2, -\text{CF}_3, -\text{COOR}^4 \) or \(-\text{CONR}^1 \text{R}^4 \), wherein \( R^4 \) is \( \text{H or (2-6C)alkyl, and R}^1 \text{ and R}^4 \) are
independently \( \text{H, (1-6C)alkyl or -(1-6C)alkylene-C}_2 \text{H}_5 \), or \( R^3 \) and \( R^6 \) together are
\( \text{(3-6C)alkylene, or X is a heterocycle selected from 2-thiazeole, 2-thiazoline, 2-benzothiazeole,} \)
2-oxazole, 2-oxazoline, 2-benzoaxazole and 2-benzimidazole, which heterocycles are
optionally substituted with one or more \( \text{(1-6C)alkyl, (1-6C)alkoxy or oxo;} \)
or a pharmaceutically acceptable salt thereof.

2. The compound of claim 1, wherein \( R^2 \) is \( \text{H, (1-12C)alkyl, (2-12C)alkenyl, benzyl or} \)
\(-\text{(1-6C)alkylene-COOH or an ester derivative thereof, R}^3 \) is \( \text{(1-12C)alkyl, (3-8C)cycloalkyl,} \)
di\( (3-8C)cycloalkylmethyl, -(1-6C)alkylene(3-8C)cycloalkyl, (6-14C)aryl,} \)
di\( (6-14C)arylmethyl, or -(1-6C)alkylene-(6-14C)aryl, wherein the cycloalkyl and aryl groups} \)
may optionally be substituted with one or more substituents selected from halogen, hydroxy, trifluoromethyl, (1-6C)alkyl or (1-6C)alkoxy; A is proline, 3,3-dimethylproline, thiazolidine-4-carboxylic acid, 4-cis-ethylproline, 3,4-dehydroproline, 2-azetidine carboxylic acid, pipecolinic acid, octahydroindole-2-carboxylic acid, azaproline, 2-amino isobutyric acid or valine, and X is -COOR⁴, -CONR⁵R⁶, 2-thiazole, 2-thiazoline, 2-benzothiazole, 2-oxazole, 2-oxazoline, 2-benzoxazole or 2-benzimidazole.

3. The compound of claim 1 or 2, wherein R² is -NR¹R³ and Rᵇ is R³; R¹ is -(1-6C)alkylene-COOH or an ester derivative thereof; R³ is (1-12C)alkyl, -CH₂-(3-8C)cycloalkyl, dicyclohexylmethyl, diphenylmethyl, or benzyl optionally substituted with one or two substituents selected from halogen, hydroxy or (1-6C)alkoxy; B is lysine; and X is -COOR⁴, -CONR⁵R⁶, 2-thiazole or 2-oxazole.

4. The compound of any one of claims 1-3, wherein A is proline, 3,4-dehydroproline or azetidine carboxylic acid.

5. The compound of any one of claims 1-4, wherein and R₂ is H; and X is -COOH.

6. The compound of claim 5, wherein R² is -NR¹R³ and Rᵇ is R³; R¹ is -CH₂COOH or -CH₂CH₂COOH, R₁ is -CH₂-cyclohexyl, -CH₂-cyclo-octyl, -CH₂-(para-(1-6C)alkoxy-substituted)phenyl or -(CH₂)₃CH₃; A is proline, 3,4-dehydroproline or azetidine carboxylic acid; and B is lysine.

7. The compound of claim 1, being HOOC-CH₂-D-cyclohexylalaninyl-prolinyl-lysyl-COOH or HOOC-CH₂-D-(p-methoxy-phenylalanyl)-prolinyl-lysyl-COOH.

8. A pharmaceutical composition comprising the compound of any one of claims 1-7 and pharmaceutically suitable auxiliaries.

9. The compound of any one of claims 1-7 for use in therapy.
10. Use of the compound of any one of claims 1-7 for the manufacture of a medicament for treating or preventing thrombin-related diseases.