Amino Acids (2004) 27: 19–27
DOI 10.1007/s00726-004-0099-z

Amino Acids
Printed in Austria

Synthesis and application of methyleneoxy pseudodipeptide building blocks in biologically active peptides

J. Hlaváček[1], **J. Mařík**[1], **J. Konvalinka**[1], **B. Bennettová**[2], and **R. Tykva**[1]

[1] Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Prague, Czech Republic
[2] Institute of Entomology, Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic

Received January 27, 2004
Accepted April 5, 2004
Published online July 6, 2004;

Summary. Pseudodipeptides H-Pheψ[CH_2O]Phe-OH, H-Tyrψ[CH_2O]Asp-OH and H-Proψ[CH_2O]-D-Thr-OH were synthesized using the intramolecular Williamson reaction via substituted morpholin-3-one ring with the nitrogen atom protected with bulky Boc group. This protection and the substituent at C_5 position induced the stereospecific alkylation at the C_2 position introducing the side chain of the C-terminal amino acid mimetic. In the first pseudodipeptide a quenching of the enolate with benzaldehyde was followed by dehydration and corresponding double bond was hydrogenated with high stereospecific purity. In the other pseudodipeptides, this alkylation was carried out directly by tert-butyl 2-bromoacetate or acetaldehyde. However, in the latter reaction an *R* configuration of C_3 substituent in conjugated lactame ring was determined using a NOE NMR. Consequently, after opening this ring by acidic hydrolysis, the C-terminal part of corresponding pseudodipeptide possessed the side-chain of D-Thr mimetic, contrary to former one. Synthesized pseudodipeptides were introduced into HIV protease inhibitors and into peptides with oostatic activity.

Keywords: Methyleneoxy isoster – HIV protease inhibitor – Oostatic activity – Peptide synthesis

Abbreviations: The nomenclature and symbols of amino acids follow Recommendations of IUPAC/IUB Joint Commission on Biochemical Nomenclature. Eur J Biochem (1984) 138: 9–37. Additional abbreviations include: AAA, amino acid analysis; AcOH, acetic acid; ACN, acetonitrile; Ahx, 2-aminohexanoic acid; Boc, *tert*-butoxycarbonyl; DCC, *N*,*N*-dicyclohexylcarbodiimide; DIC, *N*,*N*-diisopropylcarbodiimide; DIEA, *N*,*N*-diisopropylethylamine; DMA, *N*,*N*-dimethylacetamide; DMAP, 4-(dimethylamino)pyridine; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; EDT, ethane-1,2-dithiol; EtOAc, ethyl acetate; Et_2O, diethyl ether; EtOH, ethanol; FAB MS, fast atom bombardment mass spectrometry; Fmoc, (fluoren-1-yl-methoxy)carbonyl; HBTU, O-(benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HOBt, 1-hydroxybenzotriazole; LDA, lithium diisopropylamide; LHMDS, lithium bis(trimethylsilyl)amide; OMe, methyl ester; OSu, succinimide ester; PE, light petroleum; iPrOH, isopropanol; *t*Bu, tert-butyl; TEA, triethylamine; TFA, trifluoroacetic acid; THF, tetrahydrofurane; TIS, tri-isopropylsilane; TOTU, O-cyanoethoxycarbonylmethylidene amino-1,1,3,3-tetramethyluronium tetrafluoroborate; Trt, triphenylmethyl

1 Introduction

The replacement of peptide bond with a suitable surrogate can increase the stability of peptide toward enzymatic degradation, prolong the half-time of peptide action and improve peptides transport into a cell, Spatola (1983).

We have been interested in CH_2O and CH_2S surrogates for several years (Hlaváček et al., 1984; Hlaváček et al., 1992; Hlaváček et al., 1996; Mařík et al., 2001; Mařík et al., 2002) since they offer a polar, flexible and to proteolytic enzymes resistant structures. Contrary to CH_2S, the geometry of CH_2O surrogate is very similar to that of trans-amide bond and resembles peptide bond in extended conformation (Roubini et al., 1991; Norman et al., 1996). In addition, the latter one exhibits a negligible nucleophilicity and a resistance to oxidation.

An introduction of both the surrogates to peptide molecules can thus influence their biological activity in a different way. For example, the CH_2O significantly accelerated and enhanced biological activity of oostatic peptides contrary to the CH_2S surrogate that evoked a moderate effect only (Mařík et al., 2001). The replacement of Leu^8-Gly^9 peptide bond in oxytocin either maintained (CH_2O) its biological activities or strongly reduced (CH_2S) its blood pressure activity (Hlaváček et al., 1984; Hlaváček et al., 1992). However, the introduction of the former into oxytocin and vasopressin as the substitute for Tyr^2-Ile^3 and Tyr^2-Phe^3 bonds (Mařík et al., 2002), eliminated biological activities of both the hormones. Some modified di- and tripeptides of the C-terminal part of oxytocin containing CH_2O surrogate were able to reverse anticonvulsive shock-induced amnesia (Nicolaides et al.,

1986) and the introduction of CH_2O into molecules of substance P_{6-11} and [Leu^8]enkephalin amide resulted in agonists with selective activity and affinity to corresponding receptors (Roubini et al., 1991).

In this paper we focused our attention to the preparation of pseudodipeptides **1f**, **2d** and **3c** with CH_2O surrogate and their utilization as building blocks in the synthesis of different bioactive peptides.

In the synthesis of the pseudodipeptides we utilized the intramolecular modification of the Williamson reaction, providing stereochemically-defined compounds (TenBrink, 1987; Breton et al., 1990). According to Fig. 1, the starting compounds for the C_2-alkylation reaction, (5*S*)-5-benzylmorpholin-3-one (**1a**) and (5*S*)-5-[4-(benzyloxy)benzyl]-morpholin-3-one (**2a**), respectively, were prepared by NaH in THF mediated condensation of phenylalaninol or O-benzyltyrosinol with ethyl 2-chloroacetate which was found to be more compatible with amino group of aminoalcohols than corresponding 2-bromoderivative. Protection with di-tert-butoxydicarbonate yielded (5*S*)-5-benzyl-4-tert-butoxycarbonylmorpholin-3-one (**1b**) and (5*S*)-5-[4-(benzyloxy)benzyl]-4-(tert-butoxycarbonyl)morpholin 3-one (**2b**). The enolates, generated from **1b** or **2b** on reaction with lithium diisopropylamide were quenched with benzaldehyde or with tert-butyl bromoacetate yielding the corresponding 2,4,5-substituted morpholin-3-ones **1c** and **2c** (Fig. 2). The elimination of hydroxyl group in **1c** provided (5*S*, 2*Z*)-5-benzyl-2-benzyliden-4-tert-butoxycarbonylmorpholin-3-one (**1d**) that was hydrogenated to (2*S*, 5*S*)-2,5-dibenzyl-4-tert-butoxycarbonyl-morpholin-3-one (**1e**) in *dr* greater than 99%. The compounds **1e** and **2c** were refluxed with 6 M HCl to open the morpholin-3-one rings and pseudodipeptides H-Pheψ[CH_2O]Phe-OH and H-Tyrψ[CH_2O]Asp-OH were treated with di-tert-butyldicarbonate in the presence of 1 M NaOH to introduce

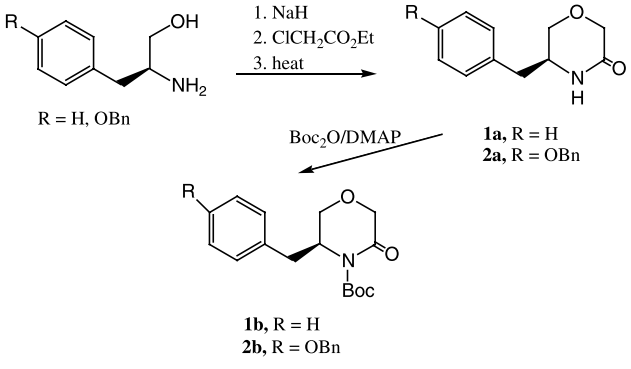

Fig. 1

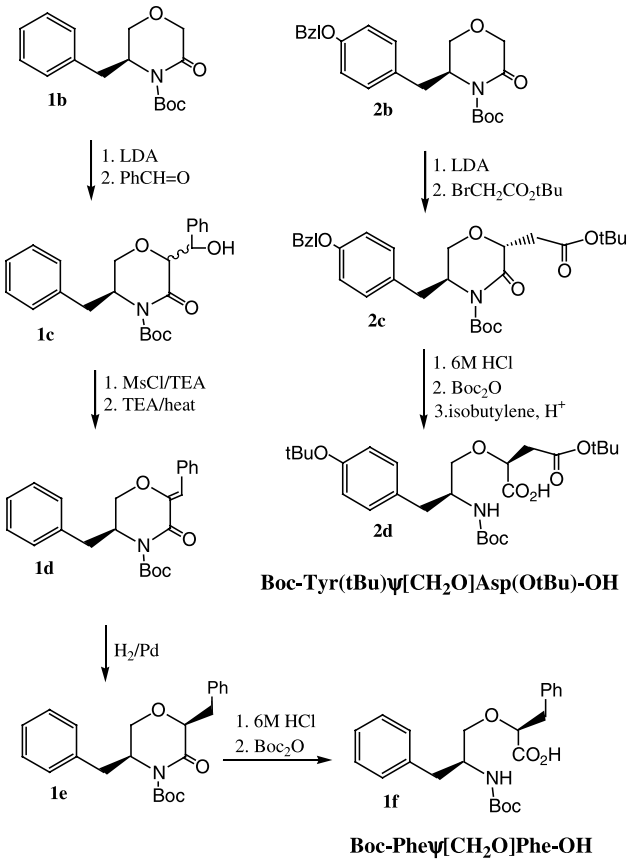

Fig. 2

N-Boc protecting groups (**1f**) and latter one consequently with isobutylene under catalysis of sulfuric acid to introduce tBu protection in the side-chains of amino acid residue mimetics, affording **2d**.

In the synthesis of pseudodipeptide **3c**, the (6*S*)-2-oxo-1-aza-4-oxabicyclo-[4.3.0]nonan (**3a**) was prepared first. This compound, on reaction with lithium bis(trimethylsilyl)amide afforded enolate, which was quenched with acetaldehyde to yield (3*R*,6*S*)-2-oxo-1-aza-3-(2-hydroxyethyl)-4-oxabicyclo-[4.3.0]nonan (**3b**). The lactam ring was opened by hydrolysis as above and the N-Fmoc protecting group was introduced to obtain pseudodipeptide building block **3c** (Fig. 3). NMR analysis has found the side-chain of the C-terminal amino acid mimetic to possess configuration of D-Thr, probably due to the pyrrolidine and morpholinone conjugated rings in the intermediate **3b**.

The synthetic potential of pseudodipeptide building blocks **1f**, **2d** and **3c** in mimetic chemistry and the effect of methyleneoxy replacement on biological properties were investigated by their introduction into molecules of selected bioactive peptides.

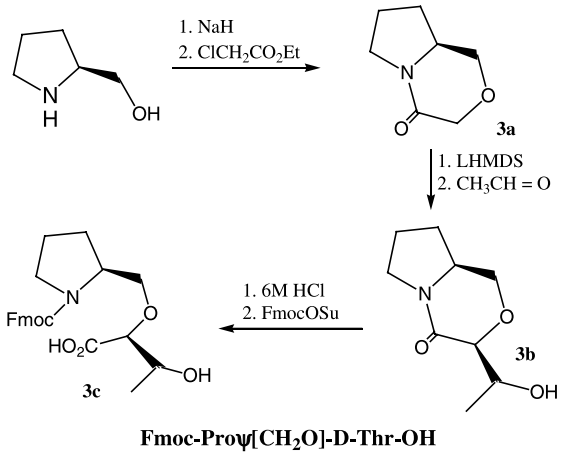

Fig. 3

Protected pseudodipeptide **1f** was utilized in the preparation of inhibitors **1i** and **1k** of HIV-1 aspartic protease (Krausslich et al., 1988; Kohl et al., 1988; Wlodawer et al., 1989). Their synthesis was carried out in solution by segment condensation of Boc-Pheψ[CH_2O]Phe-OH (**1f**) with H-Glu(OBzl)-Phe-NH_2 and H-Gln-Phe-NH_2, respectively, to yield compounds **1h** and **1k**. In the former, consequent catalytic hydrogenolysis of Bzl protecting group at glutamic acid residue was carried out.

Protected pseudodipeptides **2d** and **3c** were used for synthesis of analogs of the trypsin modulated oostatic factors isolated from mosquito *Aedes aegypti* (*Aed*-TMOF), (Borovsky et al., 1990; Borovsky et al., 1993) and from flesh fly *Neobellieria bullata* (*Neb*-TMOF), (Bylemans et al., 1994), during our program on a control of reproduction in several insect species (Hlaváček et al., 1997; Hlaváček et al., 1998). In the synthesis of pseudotetrapeptide **2f** the segment condensation of Boc-Tyr(*t*Bu) ψ[CH_2O]Asp(O*t*Bu)-OH (**2d**) with a dipeptide H-Pro-Ala-OMe (Hlaváček et al., 1998), mediated by TOTU with HOBt in DMF solution, in the presence of DIEA, was applied. This coupling was followed by treatment of the protected intermediate **2e** with TFA-TIS mixture and saponification of methyl ester with yield of corresponding pseudotetrapeptide **2f**.

The synthesis of pseudohexapeptide **3d** was carried out on solid support – Wang resin – using Fmoc protected Asn(Trt), Leu, His(Trt), Proψ[CH_2O]-D-Thr and using the protocol described in Experimental part.

Boc-Pheψ[CH_2O]Phe-X

1f, X = OH
1h, X = Glu(OBzl)-Phe-NH_2
1i, X = Glu-Phe-NH_2
1k, X = Gln-Phe-NH_2

X-Tyr(R^1)ψ[CH_2O]Asp(R^2)-Y

2d, X = Boc, R^1 = *t*Bu, R^2 = O*t*Bu Y = OH
2e, X = Boc, R^1 = *t*Bu, R^2 = O*t*Bu Y = Pro-Ala-OMe
2f, X = R^1 = R^2 = H, Y = Pro-Ala-OH

X-Proψ[CH_2O]-D-Thr-Y

3c, X = Fmoc, Y = OH
3d, X = H-Asn, Y = Asn-Leu-His-OH

2 Materials and methods

A General methodology

Protected amino acids were purchased from Bachem (Bubendorf, Switzerland) or were prepared in our laboratory following general protocols (Wünsch, 1974). Wang resin (200–400 mesh, 1% DVB, 1.3 mmol/g) was purchased from Calbiochem-Novabiochem AG (Läufelfingen, Switzerland). Solvents were evaporated *in vacuo* on a rotary evaporator (bath temperature 30°C); DMF was evaporated at 30°C and 150 Pa. Progress in solid phase synthesis was monitored by the ninhydrine test (Kaiser et al., 1970). Optical rotations were measured on a Perkin-Elmer 141 MCA polarimeter at 22°C. The samples for amino acid analysis were hydrolyzed with 6M HCl containing 3% of phenol at 110°C for 20 h. The amino acid analyses were performed on Biochrom 20 instrument (Pharmacia, Sweden). Molecular weights of the peptides were determined using mass spectroscopy with FAB technique (Micromass, Manchester, England). For HPLC a Spectra Physics instrument with an SP 8800 pump, an SP 4290 integrator and Thermo Separation Products Spectra 100 UV detector was used. The compounds were purified by semipreparative HPLC on a 25 × 1 cm column, 10 μm Vydac RP-18 (The Separations Group, Hesperia CA, U.S.A.), flow rate 3 ml/min, detection at 220 nm using a 0–100% gradient of ACN in 0.05% aqueous TFA, 120 min, unless otherwise stated. The analytical HPLC was carried out on a 25 × 0.4 cm column, 5 μm LiChrospher WP-300 RP-18 (Merck, Darmstadt, Germany), flow rate 1 ml/min, detection at 220 nm, using a 0–90% gradient of ACN in 0.05 aqueous TFA, 60 min.

B Pseudopeptide synthesis

(5*S*)-5-Benzylmorpholin-3-one **1a** (Fig. 1)

To a solution of phenylalaninol (5.0 g, 33 mmol) in THF (400 ml) was added NaH (0.8 g, 33 mmol) as a 60% dispersion in mineral oil (1.32 g) at room temperature. The reaction mixture was stirred for 30 min at room temperature under nitrogen, the evolution of hydrogen was observed during this period. To this mixture ethyl chloroacetate (4.04 g, 33 mmol) was added drop-wise in 5 min. The resulting mixture was stirred for 30 min at room temperature and for 3 h at reflux. Then the mixture was cooled to the room one and poured into 1 M HCl (300 ml). The mixture was extracted with EtOAc (3 × 100 ml), the collected organic extracts were washed with water, brine and dried over Na_2SO_4. The crude product was crystallized from a PE-EtOAc mixture to give 2.2 g (54%) of **1a**. M.p. 86–87°C, $[\alpha]_D$ +4.0° (c 0.66, MeOH). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.69 dd, 1H, J = 13.4, 9.2 and 2.85 dd, 1H, J = 13.5, 5.3 (C(5)–CH_2); 3.48 dd, 1H, J = 11.5, 6.6 and 3.87 dd, 1H, J = 11.6, 3.7 (C(6)H_2); 3.77 ddd, 1H (C(5)H); 4.19 dd, 1H, J = 17.9, 0.9 and 4.25 ddd, 1H, J = 17.8, 1.2 (C(2)H_2); 5.89 bs, 1H (NH); 7.16 m, 5H (C_6H_5). For $C_{11}H_{13}NO_2$ (191.2) calculated: 69.09%C, 6.85%H, 7.32%N; found: 68.88%C, 6.94%H, 7.51%N; FAB MS, m/z 192.1 ($M^+ + 1$).

(5*S*)-5-Benzyl-4-(tert-butoxycarbonyl)morpholin-3-one, **1b** (Fig. 1)

To the solution of compound **1a** (2.5 g; 13 mmol) in DCM (80 ml), di-tert-butoxydicarbonate (5.7 g; 26 mmol) and DMAP (3.2 g, 26 mmol) were added at 0°C. The mixture was stirred at room temperature for 3 h. Reaction mixture was washed with 20% citric acid (3 × 50 ml), brine and then dried over sodium sulfate. The product was purified using column chromatography to give 3.4 g (90%) of the morpholin-3-one derivative **1b** as a colorless oil. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.59 s, 9H (Boc); 3.01 dd, 1H, J = 11.9, 16.5 and 3.06 dd, 1H, J = 12.8 (C(5)–CH_2); 3.58 dt, 1H, J = 12.4, 2.3, 1.1 and 3.79 dt, 1H, J = 12.4, 1.2 (C(6)H_2); 4.21 m, 1H (C(5)H); 4.18 d, 1H, J = 17.4, 0.9 and 4.36 dt, 1H, J = 17.4, 1.1 (C(2)H_2); 7.28 m, 5H (C_6H_5). For $C_{16}H_{21}NO_4$ (291.4) found FAB MS, m/z 292.2 ($M^+ + 1$).

(5*S*)-5-Benzyl-2-hydroxyphenylmethyl-4-(tert-butoxycarbonyl) morpholin-3-one, **1c** (Fig. 2)

A solution of LDA (10 mmol) in THF (10 ml) prepared from diisopropylamine (1.78 ml; 10.4 mmol) and butyllithium (6.3 ml; 10.4 mmol, 1.6 M in hexane) at 0°C under N_2 was prepared. To this solution the morpholin-3-one derivative **1b** (2.4 g; 8.2 mmol) in THF (10 ml), cooled to −78°C, was added dropwise and the mixture was stirred for 0.5 h at this temperature. The enolate was then quenched with benzaldehyde (1.0 ml; 10 mmol) and after 1 h the solution was poured to 20% citric acid (80 ml). Resulting mixture was washed with EtOAc (3 × 80 ml), collected organic solutions were washed with brine and dried over Na_2SO_4. The crude product was purified by flash chromatography using a PE-EtOAc eluent mixture 7:3 to yield 1.2 g (37%) of compound **1c**. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.55 s, 9H (Boc); 2.68 dd, 1H, J = 11.9, 6.5 and 3.06 dd, 1H, J = 11.8, 6.4 (C(5)–CH_2); 3.58 ddd, 1H, J = 12.4, 2.3, 1.1 and 3.79 dd, 1H, J = 12.4, 1.2 (C(6)H_2); 4.18 d, 1H, J = 4.3 (OH); 4.21 m, 1H (C(5)H); 4.36 d, 1H, J = 4.6 (C(2)H); 5.27 dd, 1H, J = 4.6, 4.3 (C(2)–CH); 7.28–7.50 m, 10H (2 × C_6H_5). For $C_{23}H_{27}NO_5$ (397.3) found FAB MS, m/z 420.2 (M^+ + Na). IR ($CHCl_3$, cm^{-1}) ν_{OH} 3611, 3445; ν_{CO} 1725, 1770; $\delta_{CH(tBu)}$ 71, 1394; $\nu_{arom.}$ 1497, 1604.

(5*S*,2*Z*)-5-Benzyl-2-benzylidene-4-(tert-butoxy)morpholin-3-one, **1d** (Fig. 2)

The mesylchloride (0.35, 4.5 mmol) was added drop wise to the solution of **1c** (1.2 g, 3.0 mmol) and TEA (0.95 ml, 6.8 mmol) in DCM (20 ml) at 0°C. The reaction mixture was allowed to warm to room temperature and was stirred for 2 h after which was washed with 20% citric acid (2 × 50 ml) and brine (2 × 50 ml). The organic layer was dried over Na_2SO_4 and evaporated to dryness. The crude product was dissolved in DMF (50 ml), TEA (0.83 ml, 6.1 mmol) was added, the mixture was heated to 100°C in 30 min, cooled to room temperature and poured into EtOAc (50 ml). This solution was washed with 20% citric acid (2 × 50 ml) and brine (2 × 50 ml), organic layer dried over Na_2SO_4 and evaporated to dryness with a yield 0.8 g (73%) of **1d**. M.p. 134–135°C; $[\alpha]_D$ −70.8° (MeOH, c 0.36); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.59 s, 9H (Boc); 3.05 dd, 1H, J = 13.0, 1.9 and 3.08 dd, 1H, 13.1, 1.8 (C(5)–CH_2); 4.06 dd, 1H, J = 11.8, 1.7 and 4.15 dd, 1H, J = 11.6, 1.5 Hz (C(6)H_2); 4.23 m, 1H (C(5)H); 6.97 s, 1H (C(2)=CH); 7.28–7.51 m, 8H and 7.77 m, 2H (2 × C_6H_5). IR ($CHCl_3$, cm^{-1}) ν_{CO} 1714, 1767; $\nu_{C=C}$ 1619; $\delta_{CH(tBu)}$ 1381, 1371; $\nu_{arom.}$1493, 1605. For $C_{23}H_{25}NO_4$ (379.2) calculated: 72.81%C, 6.64%H, 3.69%N; found: 73.07%C, 6.75%H, 3.52%N; FAB MS, m/z 379.9 ($M^+ + 1$).

(2*S*,5*S*)-2,5-Dibenzyl-4-(tert-butoxycarbonyl)morpholin-3-one, **1e** (Fig. 2)

To a solution of **1d** (0.8 g, 21 mmol) in MeOH (300 ml) was added Pd – sponge and the reaction was run at room temperature and at atmospheric pressure of H_2 for 3 h. The catalyst was removed by filtration and the solution evaporated to give 0.8 g (99%) of **1e** as colorless oil. $[\alpha]_D$ −65.4° (MeOH, c 0.36); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.56 s, 9H (Boc); 2.26 dd, 1H, J = 13.1, 10.8 Hz and 2.63 ddd, 1H, J = 12.8, 4.2, 1.5 (C(5)–CH_2); 3.24 dd, 1H, J = 14.0, 4.3 and 3.28 d, 1H, J = 14.0, 5.7 (C(2)–CH_2); 3.55 ddd, 1H, J = 12.3, 2.5, 1.5 and 3.68 (dd, 1H, J = 12.4, 1.3 (C(6)H_2); 4.06 m, 1H, J = 10.7, 4.2, 2.5, 1.2 (C(5)H); 4.41 dd, 1H, J = 4.4, 5.7 (C(2)H); 7.25–7.32 m, 10H (2 × C_6H_5). For $C_{23}H_{27}NO_4$ (379.4) found FAB MS, m/z 380.2 ($M^+ + 1$).

Boc-Pheψ[CH_2O]Phe-OH, **1f** (Fig. 2)

A solution of **1e** (0.9 g, 2.3 mmol) in 6M HCl (25 ml) was heated to reflux for 3 h, cooled to room temperature and evaporated to give 0.76 g (98%) of H-Pheψ[CH_2O]-Phe-OH. HCl. M.p. 132–135°C; ^{1}H NMR (D_2O, 200 MHz) δ 2.86 d, 2H, J = 7.6 (β-H_2(Phe); 2.96 dd, 1H, J = 14 and 3.18 dd, 1H, J = 14, 4.3 (β-H_2 (Phe)); 3.34 dd, 1H, J = 10.5, J = 4.4 and 3.61 dd, 1H, J = 10.7, 3.0 (ψ-CH_2O); 4.23 dd, 1H, J = 8.2, 4.2 and 4.52 dd, 1H, J = 8.3, 4.3 (2 × α-H (Phe)); 7.30–7.50 m, 10H (2 × C_6H_5). To the solution of above hydrochloride (0.45 g, 1.5 mmol) in dioxane (3 ml) and water (1 ml) was added di-tert-butoxydicarbonate (0.39 g, 1.8 mmol) in dioxane (5 ml) at pH 8.5 adjusted by 1M NaOH, at 0°C. The mixture was stirred for 3 h at room temperature, dioxane was evaporated and aqueous phase was washed with Et_2O. After pH was adjusted to 3 with 20% citric acid the product was washed out to EtOAc (3 × 20 ml). The collected organic solutions were washed with brine (3 × 20 ml), dried over Na_2SO_4 and evaporated to dryness with a yield 0.44 g (73%) of **1f** as a colorless oil. $[\alpha]_D$ −25.9° (MeOH, c 0.45); HPLC peak retention time 42.6 min (0–90% ACN in 0.05% TFA, 60 min); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.43 s, 9H (Boc); 2.72 d, 2H, J = 7.7 (β-H_2(Phe)); 2.97 dd, 1H, J = 15.4, 9.3 and 3.19 dd, 1H, J = 14.0, 3.7 (β-H_2(Phe)); 3.43 dd, 1H, J = 9.8, 4.0 and 3.70 dd, 1H, J = 10.7, 3.0 (ψ-CH_2O), 4.35 m, 1H, J = 8.2, 4.2 and 4.62 dd, 1H, J = 8.3, 4.3 (2 × α-H(Phe)); 6.8 bs, 1H (NH); 7.26–7.45 m, 10H (2 × C_6H_5). For $C_{23}H_{29}NO_5$ (399.3) found FAB MS, m/z 400.2 ($M^+ + 1$).

Boc-Glu(OBzl)-Phe-NH_2, **1g**

A solution of Boc-Glu(OBzl)-OH (0.5 g, 1.5 mmol), DCC (0.33 g, 1.6 mmol) and HOBt (0.24 g, 1.7 mmol) in DMF (3 ml) was stirred at 0°C for 30 min, and H-Phe-NH_2 (1.5 mmol) in DMF (3 ml) was added. The mixture was stirred overnight at room temperature, dicyclohexylurea was removed by filtration and DMF was evaporated. The residue was dissolved in EtOAc, the solution washed with 1M $NaHCO_3$ (3 × 20 ml), 20% citric acid (2 × 20 ml), brine (2 × 20 ml) and was dried over Na_2SO_4. After the solution was evaporated to dryness, the resulting white solid was crystallized from EtOAc-Et_2O mixture to give 0.46 g (63%) of the protected dipeptide **1g**. M.p. 142–143°C; $[\alpha]_D$ −35.2° (c 0.3, MeOH); HPLC peak retention time (0–90% ACN in 0.05% TFA, 60 min) was 40.8 min. For $C_{26}H_{33}N_3O_6$ (483.4) found: 64.58%C, 6.88%H, 8.69%N; calculated: 64.72%C, 6.79%H, 8.49%N; FAB MS, m/z 484.0 ($M^+ + 1$).

Boc-Pheψ[CH_2O]Phe-Glu(OBzl)-Phe-NH_2, **1h**

The compound **1g** (0.24 g, 0.5 mmol) was dissolved in TFA (5 ml) and the solution was stirred for 30 min at room temperature. TFA was evaporated and the residue was triturated with Et_2O, dissolved in DMF (5 ml) and **1f** (0.2 g, 0.5 mmol), HBTU (0.19 g, 0.5 mmol), HOBt (0.067 g, 0.5 mmol) and DIEA (0.17 ml, 1 mmol) were added. The pH of reaction mixture was adjusted to 8 by DIEA and the reaction mixture was stirred at room temperature overnight. The DMF was evaporated, the solid residue was placed in the sintered glass funnel and was triturated with 1M $NaHCO_3$ (3 × 20 ml), 20% citric acid (2 × 20 ml), water 2 × 20 ml) and was dried *in vacuo* to give 0.29 (76.8%) of **1h** as a white amorphous solid. M.p. 205–207°C; $[\alpha]_D$ −13.4° (c 0.53, DMF); HPLC peak retention time (50–90%

ACN in 0.05% TFA, 20 min) was 19.8 min. For $C_{44}H_{52}N_4O_8$ (764.8) calculated: 69.09%C, 6.85%H, 7.32%N; found 68.85%C, 6.92%H, 7.41%N; FAB MS, m/z 765.6 ($M^+ + 1$).

Boc-Pheψ[CH_2O]Phe-Glu-Phe-NH_2, **1i**

To a solution of **1h** (0.35 g, 0.5 mmol) in a mixture DMF/MeOH 1:1 (200 ml) was added Pd/sponge (10 mg) and the reaction mixture was run at room temperature and atmospheric pressure of H_2 for 4 h. The catalyst was removed by filtration and the solution evaporated to dryness to give colorless oil, which was dissolved in EtOAc and was dried over Na_2SO_4. After evaporation of EtOAc 0.31 g (88%) the crude product **1i** was obtained as a white solid, which was purified by preparative HPLC. M.p. 156–159°C; $[\alpha]_D$ −35.7° (c 0.35, DMF); HPLC peak retention time (0–90% ACN in 0.05% TFA, 60 min) was 10.3 min; ^{1}H NMR (DMSO, 200 MHz) δ 1.30 s, 9H (Boc), 1.55–1.80 m, 2H (β-H_2(Glu)); 2.11 t, 2H, J = 8.6 (γ-H_2(Glu)); 2.5–3.40 m, 6H (3 × (β-H_2(Phe)); 3.65 dd, 1H, J = 9.7, 4.1 and 3.94 dd, 1H, J = 7.9, 4.0 (ψ-CH_2O); 4.25 dd, 2H, J = 8.2, 4.2 (2 × α-H(Phe)), 4.43 dd, 2H (α-H(Phe) + α-H(Glu)); 6.73 d, 1H, J = 8.2 and 6.97 d, 1H, J = 6.1 (PheNH_2); 7.15–7.28 m, 15H (3 × C_6H_5); 7.44 s, 1H (OCONH); 7.93 dd, 2H, J = 13.6, 8.7 (2 × CONH); For $C_{37}H_{46}N_4O_8$ (674.8) calculated: 65.86%C, 6.87%H, 8.30%N; found 65.98%C, 6.82%H, 7.12%N; FAB MS, m/z 675.6 ($M^+ + 1$); AAA: Glu 1.0, Phe 1.0.

Boc-Gln-Phe-NH_2, **1j**

The compound **1j** was prepared according to procedure for preparation of **1g** from Boc-Gln-OH (0.5 g, 2.0 mmol), H-Phe-NH_2 (0.33 g, 2.0 mmol), HOBt (0.33 g, 2.4 mmol) and DCC (0.46 g, 2.2 mmol). The DMF was evaporated, the remaining white solid was triturated with 1M $NaHCO_3$ (3 × 20 ml), 20% citric acid (2 × 20 ml), water (2 × 20 ml) and dried to give 0.37 g (47%) of **1j** as a white amorphous solid. M.p. 183–185°C; $[\alpha]_D$ −24.48° (c 0.75, DMF); HPLC peak retention time (0–90% ACN in 0.05% TFA, 60 min) was 25.5 min. For $C_{19}H_{28}N_4O_5$ (392.4) calculated: 58.15%C, 7.19%H, 14.28%N; found: 57.91%C, 7.04%H, 14.39%N; FAB MS, m/z 393.3 ($M^+ + 1$); AAA: Glu 1.04, Phe 1.0.

Boc-Pheψ[CH_2O]Phe-Gln-Phe-NH_2, **1k**

The compound **1k** was prepared according to the procedure for preparation of **1h** from **1j** (0.24 g, 0.5 mmol), **1f** (0.21 g, 0.5 mmol), HBTU (0.19 g, 0.5 mmol), HOBt (0.06 g, 0.5 mmol) and DIEA (0.17 ml, 1 mmol) with yield 0.22 g (65%) of **1k**. M.p. 145–150°C; $[\alpha]_D$ −33.05° (c 0.24, DMF); HPLC peak retention time (0–90% ACN in 0.05% TFA, 60 min) was 10.4 min; ^{1}H NMR (DMSO, 200 MHz) δ 1.29 s, 9H (Boc); 1.60–1.80 m, 2H (β-H_2(Gln)); 2.00 bt, 2H, J = 8.5 (γ-H_2(Gln)); 2.6–3.4 m, 6H (3 × β-H_2(Phe)); 3.6 dd, 1H, J = 9.8, 4.0 and 3.91 dd, 1H, J = 10.7, 3.0 (ψ-CH_2O); 4.25 dd, 2H, J = 8.2, 4.2 (2 × α-H(Phe)); 4.43 dd, 2H, J = 8.3, 4.3 (α-H(Phe) and (α-H(Gln)); 6.72 bd, 1H, J = 8.9 and 6.93 d, 1H, J = 5.8 (PheNH_2); 6.78 bs, 1H (NH(Gln)); 7.15–7.28 m, 16H (3 × C_6H_5 + NH(Gln)); 7.44 s, 1H (OCONH); 7.96 dd, 2H (2 × CONH). For $C_{37}H_{47}N_5O_7$ (673.8) calculated: 65.96%C, 7.03%H, 10.39%N; found: 66.12%C, 6.96%H, 10.22%N; FAB MS, m/z 674.6 ($M^+ + 1$); AAA: Glu 0.97, Phe 1.0.

(5*S*)-5-[4-(Benzyloxy)benzyl]morpholin-3-one, **2a** (Fig. 1)

To the solution of O-benzyltyrosinol (5.0 g; 20 mmol) in THF (300 ml) was added NaH (0.48 g, 20 mmol) as 60% dispersion in mineral oil (0.8 g) under a nitrogen atmosphere and at room temperature. The mixture was stirred 30 minutes and hydrogen evolution was observed during this period. To this mixture ethyl chloroacetate (2.44 g; 20 mmol) was added drop wise in 5 min. The resulting mixture was stirred 30 min at room temperature and then 3 h at reflux. The mixture was cooled to the room temperature and poured into 1N HCl (300 ml). The mixture was extracted with EtOAc (3 × 100 ml) and the collected organic extracts were washed with brine and were dried over sodium sulfate. The crude product was crystallized from mixture PE-EtOAc to give 2.2 g (51%) of the compound **2a**. M.p. 120–123°C; $[\alpha]_D$ −5.2° (MeOH, c 0.3); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.62 dd, 1H and 2.85 ddd, 1H (C(5)–CH_2); 3.55 dd, 1H and 3.94 ddd, 1H (C(6)CH_2); 3.72 m, 1H, (C(5)H); 4.19 d, 1H and 4.22 d, 1H (C(2)H_2); 5.06 s, 2H (CH_2(Bzl); 5.78 bs, 1H (NH); 6.95 m, 2H and 7.26 m, 2H (C_6H_4); 7.30–7.50 m, 5H (C_6H_5). For $C_{18}H_{19}NO_3$ (297.4) calculated: 72.71%C, 6.44%H, 4.71%N; found: 72.49%C, 6.49%H, 4.86%N; FAB MS, m/z 298.3 ($M^+ + 1$).

(5*S*)-5-[4-(Benzyloxy)benzyl]-4-(tert-butoxycarbonyl)morpholin-3-one, **2b** (Fig. 1)

To the solution of compound **2a** (2.0 g; 6.7 mmol) in DCM di-tert-butyloxydicarbonate (4.5 g; 20 mmol) and N,N-dimethylaminopyridine (2.5 g, 20 mmol) were added at 0°C. The mixture was stirred at room temperature for 3 h. Reaction mixture was washed with 20% citric acid (3 × 50 ml), brine and then dried over sodium sulfate. The crude product was purified using column chromatography to give 1.6 g (67%) of the morpholin-3-one derivative **2b**. M.p. 81–83°C; $[\alpha]_D$ −2.2° (MeOH, c 0.46); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.59 s, 9H (Boc); 2.98 m, 2H (C(5)–CH_2); 3.58 dd, 1H and 3.80 ddd, 1H (C(6)H_2); 4.15 m, 1H (C(5)H); 4.25 d, 1H and 4.35 d, 1H (C(2)H_2); 5.05 s, 2H (CH_2(Bzl)); 6.92 m, 2H and 7.18 m, 2H (C_6H_4); 7.30–7.50 m, 5H (C_6H_5). For $C_{23}H_{27}NO_5$ (397.5) calculated: 69.51%C, 6.85%H, 3.52%N; found: 69.67%C, 6.72%H, 3.71%N; MS (FAB) m/z 398.4 ($M^+ + 1$).

(2*R*,5*S*)-5-[4-(Benzyloxy)benzyl]-4-(tert-butoxycarbonyl)-2-(tert-butoxycarbonylmethyl)morpholin-3-one, **2c** (Fig. 2)

A solution of LDA in THF (10 ml) prepared from diisopropylamine (0.89 ml; 5.2 mmol) and butyllithium (3.02 ml; 4.8 mmol, 1.6 M in hexane) at 0°C under N_2 was prepared. To this solution cooled to −78°C the morpholin-3-one derivative **2b** (1.6 g; 4.0 mmol) in THF (5 ml) was added drop wise and the mixture was stirred for 30 min at this temperature. The enolate was then quenched with tert-butyl bromoacetate (0.94 ml; 6.4 mmol) and after 1 h the solution was poured to 20% citric acid (50 ml). Resulting mixture was washed with EtOAc (3 × 50 ml), collected organic solutions were washed with brine and dried over sodium sulfate. The crude product was purified using column chromatography to yield 0.65 g (31%) of the compound **2c**. M.p. 103–106°C; $[\alpha]_D$ +46.1° (MeOH, c 0.57); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.46 s, 9H (Boc); 1.57 s, 9H (Boc); 2.79 dd, 1H and 2.87 dd, 1H (C(5)–CH_2); 2.93 dd, 1H and 2.99 dd, 1H (C(2)-CH_2); 3.64 dd, 1H and 3.8 dd, 1H (C(6)H_2); 4.22 m, 1H (C(5)H); 4.72 dd, 1H (C(2)H); 5.06 s, 2H (CH_2(Bzl)); 6.92 m, 2H and 7.16 m, 2H (C_6H_4); 7.30–7.50 m, 5H (C_6H_5). For $C_{29}H_{37}NO_7$ (511.6) calculated: 68.09%C, 7.29%H, 2.74%N; found: 67.88%C, 7.22%H, 2.86%N; MS (FAB) m/z 512.4 ($M^+ + 1$).

Boc-Tyr(*t*Bu)ψ[CH_2O]Asp(OtBu)-OH, **2d** (Fig. 2)

A solution of **2c** (0.32 g; 0.63 mmol) in 6M HCl (25 ml) was heated to reflux for 3 h, cooled to room temperature and evaporated to give H-Tyrψ[CH_2O]Asp-OH. HCl, m.p. 136–138°C; HPLC peak retention time 23.21 min. To the solution of this hydrochloride (0.23 g, 0.72 mmol) in dioxane (3 ml) and water (1 ml), di-tert-butoxydicarbonate (0.31 g, 1.45 mmol) in dioxane (4 ml) was added at pH 8.5 adjusted by 1M NaOH at 0°C. The mixture was stirred for 3 h at room temperature, dioxane was evaporated and aqueous phase washed with Et_2O. After pH was adjusted to 3 with 20% citric acid the product was washed out with EtOAc (3 × 10 ml). The collected organic solutions were washed with brine (3 × 10 ml), dried over Na_2SO_4 and evaporated to dryness with a yield

0.24 g of Boc-Tyrψ[CH_2O]Asp-OH. M.p. 142–144°C. HPLC peak retention time 32.12 min (0–90% ACN in 0.05% TFA, 60 min); For $C_{18}H_{25}NO_8$ (383.4); FAB MS, m/z 384.2 ($M^+ + 1$). The residue was dissolved in DCM (20 ml) and 98% H_2SO_4 (0.2 ml) with isobutylene (40 ml) were added. Mixture was shaken for 48 h in pressure bottle at room temperature, neutralized with solid $NaHCO_3$, washed with water and dried over Na_2SO_4. The crude product was crystallized from an EtOAc-PE mixture to give 0.5 g (95%) of the compound **2d**. HPLC peak retention time was 39.2 min; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.42 s, 9H (Boc); 1.43 s, 9H (Boc); 1.51 s, 9H (Boc); 2.76 dd and 2.79 dd, 2H (β-CH_2(Asp)); 3.45 dd and 3.53 m, 2H (β-CH_2(Tyr)); 3.87 dd 1H, J = 9.8, 4.0 and 3.99 dd, 1H, J = 10.7, 3.0 (ψ-CH_2O); 4.05 m, 1H (α-H(Tyr)); 4.25 m, 1H (α-H(Asp)); 6.77 d, 1H (NH(Tyr)); 7.07 d, 2H and 7.21 d, 2H (C_6H_4). For $C_{26}H_{34}NO_8$ (496.6) calculated: 62.88%C, 8.53%H, 2.82%N; found: 63.09%C, 8.47%H, 2.96%N; MS (FAB) m/z 497.4 ($M^+ + 1$).

Boc-Tyr(*t*Bu)ψ[CH_2O]Asp(OtBu)-Pro-Ala-OMe, **2e**

A mixture of **2d** (0.12 g; 0.24 mmol), HOBt (0.04 g; 0.3 mmol), TOTU (0.1 g; 0.3 mmol) and DIEA (51 μl; 0.3 mmol) in DMF (1 ml) was added to DMF (1 ml) solution of H-Pro-Ala-OMe trifluoroacetate (0.3 mmol). Resulting reaction mixture was stirred for 20 h at room temperature and then DMF was evaporated. The crude oily product was dissolved in EtOAc (10 ml) and this solution was washed with 1 M $NaHCO_3$ (3 × 5 ml), 20% citric acid (2 × 5 ml), brine (2 × 5 ml) and was dried over Na_2SO_4. The solvent was evaporated and the residue (0.12 g) was purified by preparative HPLC to give pure **2e** with analytical HPLC peak retention time 37.4 min. For $C_{3F}H_{56}N_3O_{10}$ (678.9) calculated: 61.92%C, 8.32%H, 6.19%N; found: 62.14%C, 8.34%H, 6.09%N; MS (FAB) m/z 679.7 ($M^+ + 1$).

H-Tyrψ[CH_2O]Asp-Pro-Ala-OH, **2f**

Protected pseudotetrapeptide **2e** (0.1 g; 0.16 mmol) was treated with TFA-anisole 9:1 mixture (2 ml) for 20 min at room temperature. The reaction mixture was evaporated and ester was dissolved in water (1.2 ml) – acetone (3.9 ml) mixture and hydrolyzed by 2 M NaOH (0.32 ml) for 2 h at room temperature. The acetone was evaporated, the aqueous phase was diluted with water and washed with EtOAc (3 × 5 ml). pH was adjusted to 3 by 1 M HCl and acidic solution was applied on the column of Dowex 50 × 2 in water. After elution of chloride ions the product was eluted by 3% NH_4OH, ammonia evaporated and remaining solution lyophilized. Preparative HPLC afforded 42 mg of pure pseudotetrapeptide **2f** with analytical HPLC peak retention time 10.9 min. For $C_{21}H_{28}N_3O_7$ (450.5) calculated: 56.00%C, 6.26%H, 9.33%N, found: 55.84%C, 6.36%H, 9.51%N; FAB MS, m/z 451.4 ($M^+ + 1$); AAA: Ala 0.96, Pro 1.0.

(6*S*)-2-Oxo-1-aza-4-oxabicyclo-[4.3.0]nonan, **3a** (Fig. 3)

The compound was prepared according to the procedure for preparation of **1a** by using prolinol (4.1 g, 40 mmol), NaH (0.96 g, 40 mmol) as the 60% dispersion in mineral oil (1.6 g) and ethyl chloroacetate (4.3 ml, 40 mmol). The crude product was crystallized from PE to yield 1.75 g (30%) of **3a** as a white solid. M.p. 53–59°C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.39 m, 1H and 2.05 m, 1H (C7(CH_2)); 1.85 m, 1H and 2.05 m, 1H (C8(CH_2)); 3.24 dd, 1H and 4.14 m, 1H (C5(CH_2)); 3.52 m, 1H and 3.66 m, 1H (C9(CH_2)); 4.18 dd, 1H (C6(CH)); 4.21 dd, 1H and 4.26 dd 1H (C3(CH_2)). For $C_7H_{11}NO_2$ (141.2) calculated: 59.56%C, 7.85%H, 9.92%N; found: 59.74%C, 7.89%H, 9.71%N; FAB MS, m/z 142.1 ($M^+ + 1$).

(3*R*,6*S*)-2-Oxo-1-aza-3-(2-hydroxyethyl)-4-oxabicyclo-[4.3.0]nonan, **3b** (Fig. 3)

To a solution of **3a** (0.24 g, 1.7 mmol) in THF (10 ml), LHMDS (2 ml, 2 mmol) was added drop wise at −78°C. The resulting yellow solution was stirred at this temperature for 30 min, enolate was quenched with acetaldehyde (0.1 ml, 1.7 mmol), the reaction mixture was allowed to warm slowly to room temperature for 2 h and then poured into saturated solution of NH_4Cl (15 ml). The product was washed with $CHCl_3$ 5 × 10 ml, dried and solvent evaporated to give 0.19 g (58%) of **3b** as a yellow oil. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.26 dd, J = 6.5 and 1.28 dd, J = 6.2, 3H (CH_3); 1.4 m, 1H and 2.05 m, 1H (C7(CH_2)); 1.86 m, 1H and 2.05 m, 1H (C8(CH_2)); 3.10 d, 1H, J = 9.5 (OH); 3.33 dd, 1H, J = 11.4, 10.1 and 4.22 dd, 1H, J = 11.4, 3.9 (C5(CH_2)); 3.52 m, 1H and 3.67 m, 1H (C9(CH_2)); 3.70 m, 1H (C6(CH)); 4.02 d, 1H, J = 3.2, 0.5 (C3(H)); 4.17 m, 1H (CH). ^{13}C NMR ($CDCl_3$ 125.7 MHz, APT) δ 18.94, 18.96 (CH_3), 22.32 (C(8)H_2), 28.76, 28.68 (C(7)H_2), 44.82, 44.76 (C(9)H_2), 57.14, 57.33 (C(6)H), 68.02, 68.48 (CH), 68.63 (C(5)H_2), 78.85, 78.95 (CH). For $C_9H_{15}NO_3$ (185.2) found FAB MS, m/z 186.1 ($M^+ + 1$). IR ($CHCl_3$, cm^{-1}) ν_{OH} 3415, 3584; $\nu_{C=C}$ 1635.

Fmoc-Proψ[CH_2O]-D-Thr-OH, **3c** (Fig. 3)

A mixture of **3b** (0.18 g, 1 mmol) and HCl (5 ml) was heated in sealed tube to 100°C for 5 h. Then the reaction mixture was cooled to room temperature and evaporated to dryness to give 0.2 g (83%) of H-Proψ[CH_2O]-D-Thr-OH. HCl as a yellow oil. For $C_9H_{18}ClNO_4$ (239.8) found FAB MS, m/z 204.1 ($M^+ + 1$) corresponding to the mass without HCl. To the suspension of pseudodipeptide hydrochloride (0.2 g, 0.83 mmol) in water (12 ml), Fmoc-OSu (0.31 g, 0.92 mmol) in acetone (14 ml) was added at 0°C and the reaction mixture was stirred for 3 h at room temperature while pH was maintained at 8.5 using 10% Na_2CO_3. The dioxane was evaporated, aqueous solution was washed with Et_2O (3 × 15 ml) and acidified to pH 3 with the 1 M HCl. The product was taken up with EtOAc (3 × 15 ml), collected organic solutions were washed with brine (3 × 15 ml), dried and evaporated to yield 0.3 g (86%) of **3c**, which was purified by HPLC (0–90% ACN in 0.05% TFA, 60 min) with retention time 38.3 min. For $C_{24}H_{27}NO_6$ (425.5) calculated: 67.75%C, 6.40%H, 3.29%N; found: 67.91%C, 6.32%H, 3.12%N; FAB MS, m/z 426.3 ($M^+ + 1$).

H-Asn-Proψ[CH_2O]-D-Thr-Asn-Leu-His-OH, **3d**

The pseudohexapeptide **3d** was prepared on the Wang resin by using Fmoc protected Asn(Trt), Leu, His(Trt) and Proψ[CH_2O]-D-Thr. Deprotection of the Fmoc group was performed by 20% piperidine in DMA (10 ml, 2 × 20 min), washing with DMA (3 × 10 min) followed by coupling of corresponding protected Fmoc-amino acids (3 eq) mediated by DIC (3 eq), HOBt (3 eq) and DIEA (10 μl) in DMA (10 ml) for a few hours at room temperature, until Kaiser test was negative. The methyleneoxy pseudodipeptide building block (1 eq) was coupled using TOTU (1 eq), HOBt (0.5 eq) and DIEA (2 eq). After each coupling reaction the washing with DMA (3 × 10 ml), iProOH (3 × 10 ml), DMA (3 × 10 ml) was carried out. The deprotection and cleavage of the pseudohexapeptide from the resin were performed simultaneously with a TFA (94%), water (2.5%), EDT (2.5%), TIS (1%) mixture for 1.5 h. The resin was washed with TFA and DCM, the solvents were evaporated and the crude product was precipitated on addition of Et_2O, dissolved in water and lyophilized. The product was purified by HPLC to yield 20 mg of above pseudohexapeptide. M.p. 127–130°C; HPLC peak retention time in a gradient of 0–90% MeCN in 0.05% TFA, 60 min, was 11.49 min. For $C_{29}H_{47}N_9O_{10}$ (681.8) found FAB MS, m/z 682.5 ($M^+ + 1$). Amino acid analysis: Asp 1.9, Leu 0.9, His 1.

C Measurement of HIV-1 protease inhibition activity

Inhibition constants were determined *in vitro* by a spectrophotometric assay with the chromogenic substrate Lys-Ala-Arg-Val-Ahx-Nph-Glu-Ala-Ahx-NH_2 (Richards et al., 1990), in the mixture of sodium acetate (100 mM), NaCl (0.3 mM), substrate (28 μM) and HIV-1 protease (3.5 nM) at pH 4.7 (Majer et al., 1993).

D Insect oostatic activity assay

Peptides were separately injected in Ringer solution (2 μmol/ml, 5 μl per female) into the upper part of the thorax of Et_2O-anestetized, 24 h old females of *Neobellieria bullata* (Diptera). Treated female flies were placed into cages with untreated males and dissected at regular time intervals (on days 1, 2, 8 and 15 after application) and their ovaries were examined for changes in egg development during the first gonotrophic cycle. Egg hatchability and resorption were determined on deposited eggs in the uterus. Ovaries undergoing the second gonotrophic cycle were also examined. Histological preparations made of suspect ovaries of both gonotrophic cycles were prepared using standard procedures. They were stained with Mallory's or Mayer's hematoxyline. Differences in the degree of inhibition of correct egg development were evaluated with regard to the regular time intervals after peptide application and the type of isosteric molecule.

3 Results and discussion

Syntheses of the three protected isosteric pseudodipeptides **1f**, **2d** and **3c** were carried out using cyclic modification of Williamson etherification reaction that utilized suitably substituted morpholin-3-one ring. This substitution allowed stereospecific introduction of C-terminal amino acid side-chain mimetic derived from phenylalanine, aspartic acid and threonine, into the position 2. The NMR analysis of compound **1e** revealed a NOE interaction between the proton attached to the carbon C_2 at 4.41 ppm with the proton attached to the carbon C_6 at 3.55 ppm which has been assigned to adopt pseudoaxial position due to the interaction with the C_5 proton at 4.06 ppm, $J = 2.5$ Hz. The stereochemistry at the C_5 is known because the starting material was L-Phe. Thus, the configuration at the carbon C_2 is *S*. It confirmed our presumption that the catalyst can approach from the *Re* face to the C_2 only because of the sterical hindrance by the benzyl moiety at the C_5 of the morpholin-3-one ring. The Boc protecting group induces the axial position of the alkyl substituent at C_5. Similarly, in the preparation of **2d** the stereoselectivity was induced by benzyl moiety at the C_5 position which adopts the pseudoaxial conformation as a consequence of minimizing of the pseudoallylic $A_{1,3}$ strain caused by N-Boc protecting group (Anthony et al., 1995). The effect of the pyrrolidine and morpholinone rings conjugation in the corresponding pseudodipeptide precursor **3b** on configuration of the substituent at C_3 position, already described in the preparation of H-Proψ [CH_2O]Ala-OH (TenBrink, 1987; Mařík et al., 2001), has also been detected in the preparation of pseudodipeptide **3c**. Protected pseudodipeptides **1f**, **2d** and **3c** were successfully introduced into molecules of bioactive peptides using conventional methods of solution and solid phase synthesis. Their biological activities were assayed and compared with those described for pseudopeptides previously prepared.

Table 1. Inhibition activity of Boc-Pheψ[X]Phe-P′$_2$-Phe-NH_2

X	P′$_2$	K_i (nm)	Ref.
CH_2NH	Glu	0.2	Urban et al. (1992) Majer et al. (1993)
(*S*)-$CH(OH)CH_2$	Glu	0.02	Konvalinka et al. (1997)
(*R*)-$CH(OH)CH_2NH$	Glu	0.1	Konvalinka et al. (1997)
CH_2O	Glu	0.5	
CH_2NH	Gln	4.1	Urban et al. (1992) Majer et al. (1993)
(*S*)-$CH(OH)CH_2$	Gln	0.02	Konvalinka et al. (1997)
(*R*)-$CH(OH)CH_2NH$	Gln	140	Konvalinka et al. (1997)
CH_2O	Gln	470	

HIV protease inhibitory activity of pseudotetrapeptides **1i** and **1k** (Table 1) was found to exhibit extreme dissociation with regard to the presence of either Glu or Gln residues in position P′. It is in contrast with other isosteric couples containing other surrogates in place of peptide bond. For example, the strongest HIV protease inhibitor containing hydroxyethylene bond exhibits no dissociation of inhibitory activity with regard to the presence of both the Glu and Gln residues (Konvalinka et al., 1997).

In the field of peptides with potential oostatic activity, preliminary morphological and histological assay of pseudotetrapeptide **2f** showed devastating changes in ovarian development after its application to flesh fly *Neobellieria bullata* (Fig. 4). Morphologically affected egg chambers had irregular appearance and their shape was distorted. Histological evaluation revealed very frequent changes throughout the ovary. The changes appeared mostly in the development of the second batch of eggs (the second gonotrophic cycle); the changes in the first gonotrophic cycle were manifested by decreased hatchability of eggs in the uterus. In general, the oostatic effect of **2f** was comparable with that of corresponding non-isosteric tetrapeptide H-Tyr-Asp-Pro-Ala-OH (morphological changes in ~80% of ovaries were observed). However, the effect was lower than that of pseudotetrapeptide with CH_2O surrogate replacing Pro-Ala peptide bond in above tetrapeptide (~90% of ovaries were affected), (Hlaváček et al., 1990).

On the other hand, the introduction of pseudodipeptide building block H-Proψ[CH_2O]-D-Thr-OH into the molecule of oostatic hexapeptide isolated from flesh fly

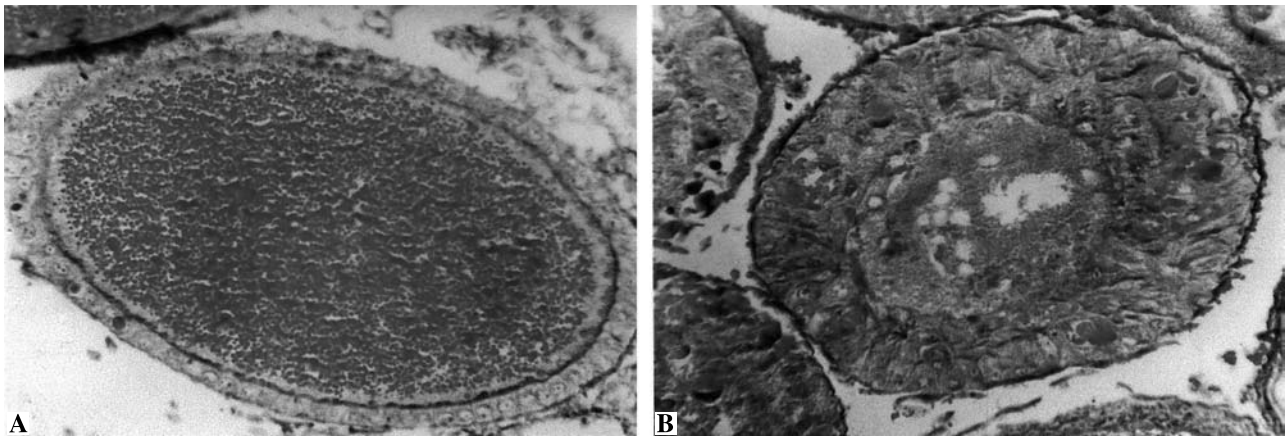

Fig. 4. Degenerative changes in female reproductive system of *Neobellieria bullata* after application of oostatic peptides. **A** Normal development of egg chamber (yolk deposition in oocyte, intact nutritive cells). **B** Egg chamber filled up with nuclei and cells originated from follicular epithelium

Neobellieria bullata exhibited a zero effect on a regulation of egg development in this insect species and thus the corresponding pseudohexapeptide **3d** was quite inactive. A different mode of the biological action in this ovarian system, described elsewhere and/or the presence of D- instead of L-Thr mimetic structure has been taken in consideration.

Results presented in this paper support suggestions described earlier that the substitution of corresponding peptide bond with methyleneoxy surrogate can influence environmental non-covalent interactions of corresponding pseudopeptide with consequences for its space structure, recognition by corresponding receptor and biological potency, as well.

Acknowledgement

This research was carried out under the research project Z4 055 905 and was supported by Grant Agency of the Czech Republic (grant No. 203/98/1330 and 203/02/0247).

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Authors' address: Dr. Jan Hlaváček, Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo nám. 2, 166 10 Prague 6, Czech Republic,
Fax: 220183573, E-mail: honzah@uochb.cas.cz