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SYNTHESIS OF ISOSTERIC METHYLENE-OXY PSEUDODIPEPTIDE ANALOGUES AS NOVEL AMIDE BOND SURROGATE UNITS

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Abstract - The syntheses of several fully protected dipeptide isosteres which incorporate a methylene-oxy bond replacing the amide bond are described. The novel methylene-oxy modification offers a polar, flexible, proteolytically resistant peptide bond surrogate which can be easily incorporated into biologically active peptides. The standard geometries of the trans-amide, methylene-oxy and methylene-thio units are compared, showing a very close geometrical resemblance of the $\psi[CH_2\text{-}O]$ unit to the amide bond.

Introduction

In the last few years there is a growing interest in the possible structural transformation of biologically active peptides, and particularly among them the increasing number of newly discovered neuropeptides[1], into non-peptidic peptidomimetic compounds as a new class of drugs[2,3]. The development of peptides as potential therapeutic agents is clearly limited by their rapid metabolism and poor transport properties through biomembranes, as well as by their wide spectrum of biological activities. A possible way to overcome these serious limitations is suggested by the most unique example provided by nature itself. The existence side by side of the opioid peptides and their non-peptidic counterparts, the opiate alkaloids, two types of natural compounds which differ in their structures, but interact similarly with a common receptor inducing identical physiological and pharmacological effects[4], clearly demonstrates that the modification of a linear peptide into a non-peptidic ligand is a desired and feasible approach. The transformation of a biologically active peptide into a non-peptidic peptidomimetic compound by a rational design of tailor-made chemical structures presents a tremendous challenge to the medicinal chemist. Replacement of the amide bonds in the peptide backbone with amide bond surrogates[3] is clearly an important tool toward such transformations, and particularly in the elucidation of the role of certain amide bonds in receptor binding and induction. Several isosteric modifications have been introduced into biologically active peptides as amide bond surrogates, these include: $\psi[CH_2\text{-}S]$[5,6], $\psi[NH\text{-}CO]$[7,8], $\psi[CH_2\text{-}CH_2]$, $\psi[CH_2\text{-}NH]$[9], $\psi[CO\text{-}CH_2]$[10], $\psi[(E)$ or (Z) $CH{=}CH]$[11,12]. One goal of these modifications is to achieve maximal topographical equivalence with the trans-amide bond. A close approximation in geometrical terms has been obtained with the $\psi[(E)$ $CH{=}CH]$ isosteric modification[12].

Introduction of amide bond surrogates may serve as:

1. Conformational probes by conferring variable degrees of rotational freedom in the backbone. 2. Change patterns of intra- and intermolecular hydrogen bond formation. 3. Modify local as well as total polarity and hydrophobicity. Some advantages resulting from the above mentioned modifications can be the enhancement of metabolic stability, increase in selectivity towards subtypes of receptors, changes in agonistic vs. antagonistic activities as well as alterations in

pharmaco-kinetic properties of the peptides such as: increased oral bioavailability, prolonged duration of action and improved penetration into the CNS.

Discussion

The methylene-oxy modification $\psi[CH_2\text{-}O]$ reported in this paper offers a polar, flexible, proteolytically resistant surrogate to the amide bond. The standard geometries of the trans-amide and $[CH_2\text{-}O]$ moieties as well as the rotational energies about the [CO-NH] and $[CH_2\text{-}O]$ bond are displayed in Fig. (1). For comparison the geometry of the analogous methylene-thio $[CH_2\text{-}S]$ peptide bond surrogate is also shown in Fig. (1).

A close examination of the standard geometries reveals for example, that the calculated $C_\alpha^i - C_\alpha^{i+1}$ distances of the $[CH_2\text{-}O]$ pseudodipeptide units are very close to that found in unmodified dipeptides (3.8 Å) whereas for the thioether $[CH_2\text{-}S]$ units this distance is increased, up to 4.2 Å, thus causing a greater distortion in the latter modification compared to the methylene-oxy unit[13,14]. Furthermore, due to reduced electronegativity and increased size of the sulfur atom the $[CH_2\text{-}S]$ modification is less polar, thus inferior as hydrogen bond acceptor compared to the $[CH_2\text{-}O]$ modification. Thus, the negligible nucleophilicity,the resistance to oxidation and its close geometrical resemblance to the amide bond, in the extended conformation, turns the $[CH_2\text{-}O]$ modification into a more desirable surrogate to the amide bond compared to the sulfur analog.

In this paper we report the synthesis of six novel fully protected methylene-oxy $[CH_2\text{-}O]$ pseudodipeptide analogs (Fig. 2), derived from the sequences of the neuropeptides substance P and enkephalin.

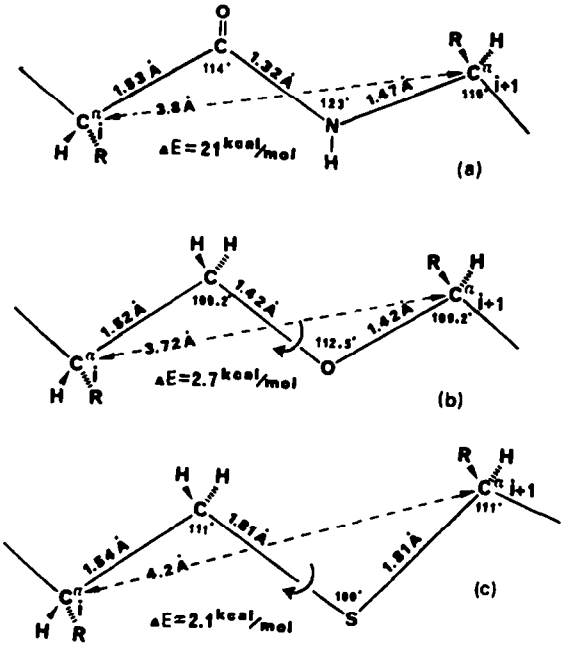
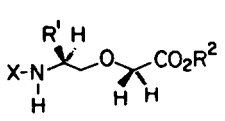


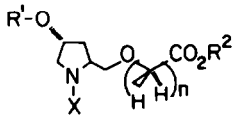
Fig. 1. Standard geometries at the extended conformation of the amide bond, $[CH_2\text{-}O]$ and $[CH_2\text{-}S]$ pseudopeptide modifications, (a), (b) and (c), respectively [13,14].

In a recent preliminary report, Tinney et al.[15] presented an alternative route for the synthesis of pseudodipeptides containing the methylene-oxy modification, which involves the acid hydrolysis of the amide bond in a pyrano lactam derivative to yield the unprotected methylene-oxy pseudodipeptide unit. Ondetti et al.[16] have incorporated the methylene-oxy modification, in some angiotensin converting enzyme inhibitors, which displayed significant activity.

However, no detailed information is available on the synthesis and characterization of such analogs.



A



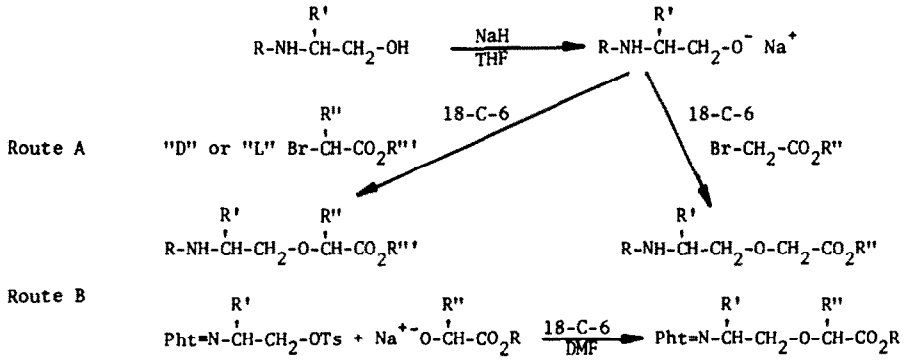
B

		X	R^1	R^2	n
	Structure A				
1	Phth-Gly ψ (CH_2O)Gly-OBu^t		H	Bu^t	-
3	Z-Tyr(Bu^t)ψ(CH_2O)Gly-OBu^t	BzlO-CO	4-Bu^{t}O-C_6H_4-CH_2	Bu^t	-
5	Z-Tyr(Bzl)ψ(CH_2O)Gly-OBu^t	BzlO-CO	4-Ø-CH_2O-C_6H_4-CH_2	Bu^t	-
7	Boc-Phe ψ (CH_2O)Gly-OEt	BzlO-CO	Ø-CH_2	Et	-
	Structure B				
10	Boc-trans-L-Hyp(Bzl)ψ(CH_2O)Gly-OEt	Bu^{t}O-CO	Ø-CH_2	Et	1
11	Boc-trans-L-Hyp(Bzl)ψ(CH_2O)βAla-OEt	Bu^{t}O-CO	Ø-CH_2	Et	2

Figure 2. Six protected pseudodipeptidic units of the general formula X-Aaaψ(CH_2-O)Gly-OR or X-Aaaψ(CH_2-O)βAla-OR

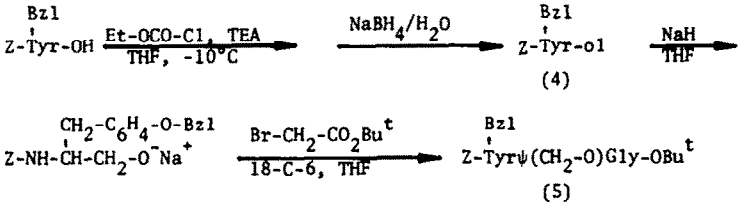
We have explored two synthetic routes to obtain the ψ[CH_2-O] modification (See Scheme 1). Route A is particularly suitable for the preparation of units of the type Aaaψ[CH_2-O]Gly (analogs 1-10) or Aaaψ[CH_2-O]βAla (analog 11), since displacement of an α-halo acetic acid ester or 3-bromopropionic acid ester respectively by the appropriate N-protected 2-amino-ethoxide is free of any configurational effects. The synthesis of a Aaaψ[CH_2-O]-L-Bbb or a Aaaψ[CH_2-O]-D-Bbb pseudodipeptidic unit following this route must require the employment of either a (R)- or (S)-2-substituted α-halo acetic acid ester, respectively, since inversion of configuration is expected at the asymmetric carbon of the α-halo acid ester during the O-alkylation. Route B is anticipated to present a general route for the preparation of either Glyψ[CH_2-O]Aaa or any Aaaψ[CH_2-O]Bbb pseudodipeptidic unit. Upon reacting tosylates of N-protected ethanolamine derivatives with alkoxides of α-hydroxy acid esters, no change in configurational integrity of the chiral centers is expected since the reaction site does not involve an asymmetric center.

The pseudo [CH_2-O] dipeptide units of the type Aaa [CH_2-O]Gly or Aaaψ[CH_2-O]βAla presented in this work, were synthesized following route A. The desired N-protected β-amino alcohols were prepared from the appropriate N-protected L-amino acids following reduction of their "mixed carbonic-carboxylic acid anhydride" obtained via ethylchloroformate/TEA with aqueous $NaBH_4$ [17,18]. This reductive step is carried out with retention of configuration at the asymmetric carbon [19].



Scheme 1. Synthetic routes for the synthesis of $\psi[CH_2\text{-}O]$ dipeptide units.

The O-alkylation of the N-protected β-amino alcohol with the appropriate α-halo acid ester required the presence of NaH and of 18-crown-6 in order to overcome the low nucleophilicity of the alkoxide. Under these conditions, the undesired side reaction namely N-alkylation was found to be negligible and the main product obtained was the O-alkylation product. The synthetic route leading to one of the pseudo $[CH_2\text{-}O]$ dipeptide units, namely, Z-L-Tyr(Bzl)$\psi(CH_2\text{-}O)$Gly-OBu^t (5) is shown in Scheme 2.



Scheme 2. Synthesis of protected pseudodipeptide Z-L-Tyr(Bzl)$\psi(CH_2O)$Gly-OBu^t (5)

The protected β-amino alcohol derivative Z-L-Tyr(Bzl)ol (4) was obtained from the appropriate amino acid derivative Z-L-Tyr(Bzl)-OH following activation with ethyl chloroformate/TEA and reduction of the "mixed anhydride" with aqueous $NaBH_4$. Subsequently, Z-L-Tyr(Bzl)ol (4) was O-alkylated with t-butyl bromoacetate in the presence of NaH and 18-crown-6 to yield the desired fully protected methylene-oxy pseudodipeptidic unit Z-L-Tyr(Bzl)$\psi(CH_2\text{-}O)$Gly-OBu^t (5). The syntheses of the various pseudodipeptide units reported in this paper were devised so as to enable their incorporation into peptide sequences. For example, the orthogonal combination of different protecting groups on the amine, hydroxyl and carboxyl functions i.e. N^{α}-benzyloxycarbonyl/O-t-Butyl or N^{α}-t-butyloxycarbonyl/O-ethyl ester allows their selective removal leading to a high degree of versatility in the synthesis and a straightforward incorporation of the pseudodipeptide units into sequences of biologically active peptides. Thus, the above mentioned methylene-oxy pseudodipeptide units (1-11) (Fig. 2) have been successfully incorporated into analogs of the neuropeptides substance P and enkephalin which displayed considerable biological activity in vitro[20,21]. It is also expected that this novel $\psi(CH_2\text{-}O)$ isosteric modification will be applied, in future, in the design of non-peptidic peptidomimetic analogs of biologically active peptides.

Experimental Section

Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 141 with a 10 cm water jacketed cell. ^{1}H NMR spectra were carried out on ^{1}H NMR Bruker WH-300 pulsed FT spectrometer operating at 300 MHz. HPLC analysis was performed on a Tracor 950 liquid chromatograph equipped with Tracor 970A variable wavelength detector and Tracor 980A solvent programmer. Compounds were monitored at 210 nm. A Whatman 10-ODS-3 or a Merck Lichrospher C-8 column (4.6 mm i.d. x 25 cm) was used in the HPLC system. TLC was run on Polygram Sil HN-R/UV254 silica gel plates using the following solvent systems: (A) EtOAc/Hexane (1:1), (B) CH_2Cl_2/MeOH (19:1), (C) CH_2Cl_2/MeOH (9:1), (D) EtOAc/Hexane (3:7), (E) CH_2Cl_2/MeOH/AcOH (17:2:1), (F) CH_2Cl_2/MeOH/AcOH (19:0.5:0.5). TLC plates were developed with ninhydrin (Merck) 4% in n-BuOH, or fluorescamine (Fluram, Hoffman La Roche & Co., AG) 1 mg% in Acetone and/or by chlorination followed by potassium iodide/o-tolidine[22]. Elemental microchemical analyses were carried out at the Microanalytical Laboratory of the Organic Chemistry Department. Unless otherwise stated and where elemental analyses are indicated only by the symbols of the elements, analytical results were within ±0.3% of the theoretical values.

Mass spectra were recorded on Varian-Mat CH-5 mass spectrometer. Fast atom bombardment (FAB) mass spectra were taken by Prof. H. Schwartz at the Technical University in Berlin. Preparative low pressure liquid chromatography (LPLC) was run on a Michel-Miller column system (ACE Glass Inc.) using a Fluid-Metering Inc. Lab. pump SY-2-CSC and silica gel 60 (230-400 mesh, Merck) or Lichroprep RP-18 (40-63 μm, Merck). N,N-dimethylformamide (DMF) was distilled from CaH_2 under reduced pressure and redistilled from benzoic anhydride under reduced pressure. THF was distilled from $LiAlH_4$ under nitrogen and redistilled from Na-benzophenone under argon.

The amino acid derivatives Z-L-Tyr(Bzl)-OH and Z-L-Tyr(Bu^t)-OH.DCHA were purchased from Merck AG; trans-L-4-hydroxyproline and ditertbutyldicarbonate (Boc_2O) were purchased from Fluka AG. L-Phenyl-alaninol was purchased from Sigma.

Pht=Glyψ(CH_2-O)Gly-OBu^t (1)

To a stirred solution of N-(2-hydroxyethyl)phthalimide[23] 5.0 g (26.0 mmol) in dry DMF (40 mL) cooled to 0°C under N_2, was added sodium hydride (50% oily dispersion) 1.37 g (28.6 mmol) in small portions. The reaction mixture was stirred for 30 min at 0°C under N_2. To this was added dropwise tert-butyl bromoacetate 12.6 mL (78 mmol) during 5 min. The reaction mixture was stirred for 2h at 0°C and overnight at room temperature, then treated with EtOAc (5 mL) at 0°C, followed by H_2O (10 mL) and AcOH immediately to adjust the pH to 6.5-7.0. The solvent was evaporated under reduced pressure; the residue was extracted with EtOAc and washed with brine, dried over $MgSO_4$ and evaporated to dryness under reduced pressure. The crude product was further purified by means of open column chromatography on silica gel(60-230 mesh) using a gradient of EtOAc in hexane (0% to 10%) as eluant. The pure product was obtained by pooling fractions eluted with 5% EtOAc in hexane. Yield: 3.8 g (48%); m.p. 36°C; TLC: R_{fA}= 0.52, R_{fB}=0.30; HPLC (H_2O-MeOH 35:65) k'=3.25; Anal. ($C_{14}H_{19}NO_5$) C, H, N; ^1H NMR ($CDCl_3$/TMS): δ= 7.86-7.70 (m, 4H, arom.), 3.98 (s, 2H, O-CH_2-CO-), 3.95-3.91 (t, 2H, -CH_2-CH_2-O-), 3.82-3.79 (t, 2H, N-CH_2-), 1.43 (s, 9H, -Bu^t $C(CH_3)_3$); HRMS: 249 (M+H-C_4H_9)$^+$, 204 (M-$COOC_4H_9$)$^+$, 190 (M-$CH_2COOC_4H_9$)$^+$, 174 (Pht=N-CH_2-CH_2)$^+$, 160 (Pht=N-CH_2)$^+$, 145 (C_4H_9OCO-CH_2O-CH_2)$^+$, 131 (C_4H_9OCO-CH_2O)$^+$.

Z-L-Tyr(Bu^t)-ol (2)

To a stirred solution of Z-L-Tyr(Bu^t)-OH, obtained from its dicyclohexyl ammonium (DCHA) salt 7.70 g (14.04 mmol) in dry THF (40 mL) under nitrogen and at -10°C was added triethylamine 1.96 mL (14.04 mmol) followed by ethylchloroformate 1.34 mL (14.04 mmol). The reaction mixture was stirred for 30 min at -10°C, then filtered to remove triethylammonium chloride. The filtered solution was kept at -5°C and added over a period of 10 min to a solution of sodium borohydride 1.06 g (18.08 mmol) in H_2O (8 mL) at 0°C. The reaction mixture was stirred overnight at room temperature, filtered and evaporated under reduced pressure. The residue was redissolved in EtOAc and the organic phase extracted with 5% $NaHCO_3$, brine, dried over Na_2SO_4 and evaporated under reduced pressure. The crude product was further purified by means of LPLC with a silica gel column (2.5x2.5 cm, 230-4-0 mesh) using a gradient of MeOH in CH_2Cl_2 (0 to 2%). The main fraction eluted along with 2% MeOH in CH_2Cl_2, yielded 3.9 g (78%) of pure (2), which was obtained as an oil. TLC R_{fA}=0.61, R_{fB}=0.59; $[\alpha]_D^{25}$ -38.3° (C=1.0, MeOH). Anal: ($C_{21}H_{27}NO_4$) C, H, N; ^1H NMR ($CDCl_3$/TMS): δ=7.32-7.29, 5.07-6.86 (m, 9H, C_6H_5-, -C_6H_4-O-), 5.04 (s, 2H, C_6H_5-CH_2-O-), 4.96 (m, 1H, -OH), 3.92 (bs, 1H, Tyr C_αH), 3.71-3.53 (m, 2H, -CH_2-O-), 2.78 (bd, 2H, Tyr $C_\beta H_2$), 1.29 (s, 9H, -O-$C(CH_3)_3$).

Z-L-Tyr(Bu^t)ψ(CH_2-O)Gly-OBu^t (3)

To a stirred solution of (2) 2.54 g (7.11 mmol) in dry THF (50 mL) under nitrogen at 0°C was added sodium hydride (50% oily dispersion), 0.55 g (11.48 mmol). The reaction mixture was stirred for 30 min at 0°C and 18-crown-6, 1.88 g (7.11 mmol) was added followed by tertbutyl-bromoacetate 11.48 mL (71.11 mmol). The reaction mixture was stirred for 1 h at 0°C then overnight at room temperature, and was worked up in the usual manner. The crude product was further purified by means of LPLC liquid chromatography with a silica gel column (2.5x25 cm) using a gradient of EtOAc in hexane (0° to 20%). The main fractions eluted along with 10% EtOAc in hexane were pooled and the solvent evaporated under reduced pressure to give pure (3) as a pale yellow oil. Yield: 2.32 g (69%); TLC R_{fA}=0.33, R_{fB}=0.45; $[\alpha]_D^{25}$-16.5° (C=1.0, MeOH). Anal: ($C_{27}H_{37}NO_6 \cdot 0.35\ H_2O$) C,H,N; ^1H NMR ($CDCl_3$/TMS): δ = 7.39-7.33, 6.91-6.87 (m, 9H, C_6H_5-, -C_6H_4-O-), 5.50 (d, Tyr C NH), 5.08 (s, 2H, C_6H_5-CH_2-O-), 4.13 (bd, Tyr C H), 3.94-3.92 (d, 2H, -O-CH_2-CO), 3.46-3.44 (d, 2H, -CH_2-O-), 2.78 (m, 2H, Tyr $C_\beta H_2$), 1.46 (s, 9H, -CO-O-$C(CH_3)_3$), 1.32 (s, 9H, -C_6H_4-O-$C(CH_3)_3$). FAB MS: 473 $[M+H]^+$, 416 $[M-C_4H_9]^+$, 372 $[M-COOC_4H_9]^+$, 339 $[M-C_6H_5CH_2COO]^+$, 316 [372-C_4H_9]$^+$, 282 [339-C_4H_9]$^+$, 181 [316-$C_6H_5CH_2COO$]$^+$.

Z-L-Tyr(Bzl)-ol (4)

To a stirred solution of Z-L-Tyr(Bzl)-OH 3.0 g (7.40 mmol), in THF (21 ml), cooled to -10°C under N_2 was added triethylamine 1.02 mL (7.40 mmol) and ethylchloroformate 7.07 mL (7.40 mmol). The reaction mixture was stirred for 30 min at -10°C, filtered to remove triethylammonium chloride and added over a period of 15 min to a solution of sodium borohydride 0.562 g (14.80 mmol) in H_2O (6 mL) at 0°C. The reaction mixture was stirred overnight at room temperature, then evaporated under reduced pressure; the residue was taken in water (50 mL) and acidified to pH 3.0 at 0°C with 1N $KHSO_4$, extracted with EtOAc (2x150 mL). The organic phase was washed with 5% $NaHCO_3$ (5x25 mL), saturated NaCl solution, dried over $MgSO_4$ and evaporated in vacuo. The crude product was recrystallized from EtOAc/hexane, to give pure (4). Yield: 2.2 g (76%); m.p. 114-115°C; $[\alpha]_D^{25}$-31.5° (C=1.0, MeOH); TLC: R_{fA}=0.41, R_{fB}=0.30; Anal. ($C_{24}H_{25}NO_4$) C,H,N; ^1H NMR ($CDCl_3$/TMS): δ=7.44-6.88 (m, 14H, C_6H_5-, -C_6H_4-O-), 5.02 (s, 2H, C_6H_5-CH_2-O-CO-), 4.99 (s, 2H, C_6H_5-CH_2-O-C_6H_4-), 4.95 (bs, 1H, -OH), 3.86 (bs, 1H, Tyr C_αH), 3.68-3.48 (m, 2H, -CH_2-O-), 2.77 (bd, 2H, Tyr $C_\beta H_2$).

Z-L-Tyr(Bzl)ψ(CH_2-O)Gly-OBu^t (5)

To a stirred solution of (4) 1.0 g (2.55 mmol) in dry THF (20 ml) cooled to 0°C under N_2, was added sodium hydride (50% oily dispersion), 0.135 g (2.81 mmol). The reaction mixture was stirred for 1 h at 0°C under N_2, followed by 18-crown-6, 0.337 g (1.28 mmol) and a solution of tert-butyl bromoacetate 4.12 mL (25.5 mmol) in dry THF (8 mL). The reaction mixture was stirred for 1 h at 0°C and overnight at room temperature, then worked up as described previously for compound (1). The crude product was further purified by means of LPLC column chromatography on silica gel (230-400 mesh) using a gradient of EtOAc in hexane (0% to 30%) as eluant. The pure product was obtained by collecting the fraction eluted with 25% EtOAc in hexane. Yield 0.937 g (73%); $[\alpha]_D^{25}$-24.6° (C=1.0, MeOH); R_{fA}=0.67, R_{fD}=0.37; HPLC (H_2O-MeOH 30:70 and 35:65) k'=0.71 and k'=1.71 respectively. Anal. ($C_{30}H_{35}NO_6$) C,H,N; ^{1}H NMR ($CDCl_3$/TMS) δ=7.44-6.88 (m, 14H, C_6H_5-C_6H_4-O-), 5.52 (bs, 1H, Tyr C_αNH), 5.09 (s, 2H, C_6H_5-CH_2-O-CO), 5.03 (s, 2H, C_6H_5-CH_2-O-C_6H_4-), 3.95-3.92 (bd, 3H, -O-CH_2-CO, Tyr C_αH), 3.95-3.92 (bd, 3H, -O-CH_2-CO, Tyr C_αH), 3.47-3.46 (bd, 2H, -CH_2-O-), 2.85 (m, 2H, Tyr $C_\beta H_2$), 1.47 (s, 9H, -O-C(CH_3)$_3$). FAB-MS: 506 $[M+H]^+$ 371 $[M+H-C_6H_5CH_2]^+$, 314 $[371-C_4H_9]^+$, 270 $[371-C_4H_9COO]^+$, 256 $[371-C_4H_9COOCH_2]^+$, 240 $[371-C_4H_9COOCH_2-O]^+$, 223 $[314-C_6H_5CH_2]^+$.

Boc-L-Phe-ol (6)

L-phenylalaninol was reacted with di-tert-butyl-dicarbonate according to the method of Moroder et al. (24) but with the following modifications: A solution of L-phenylalaninol 5.0 g (33.1 mmol) in 99 mL of dioxane/water (2:1) and 33 mL of 1N $NaHCO_3$ was stirred at 0°C and di-tert-butyl-dicarbonate 8.0 g (36.4 mmol) was added. The reaction mixture was stirred overnight at room temperature and the solvent evaporated under reduced pressure. The aqueous phase was extracted with EtOAc (3x100 mL) washed with 2N $KHSO_4$, saturated NaCl solution, dried over $MgSO_4$ and evaporated in vacuo. The crude product was recrystallized from EtOAc/hexane. Yield: 6.7 g (80%); m.p. 94-96°C ; $[\alpha]_D^{25}$-29.5° (C=2.0, MeOH); TLC: R_{fC}=0.70, R_{fA}=0.36; Anal ($C_{14}H_{23}NO_3$) C,H,N.

Boc-L-Pheψ(CH_2-O)Gly-OEt (7).

To a stirred solution of (6) 0.65 g (2.58 mmol) in dry THF (20 mL) cooled to 0°C under N_2, was added sodium hydride (50% oily dispersion) 0.136 g (2.84 mmol). The reaction was stirred for 45 min at 0°C, followed by 18-crown-6, 0.340 g (1.29 mmol) and a solution of ethyl bromoacetate 0.425 mL (3.82 mmol) in dry THF (5 mL) was added dropwise during 5 min. The reaction mixture was stirred for 1 h at 0°C and overnight at room temperature, then worked up in a standard fashion. The crude product was further purified by means of LPLC colum chromatography on silica gel (230-400 mesh) using a gradient of EtOAc in hexane (0 to 35%) as eluant. The main fractions eluted with 7.5% EtOAc in hexane were pooled, evaporated under reduced pressure to give the pure pseudo-dipeptide derivative (7). Yield: 0.47 g (54%); m.p. 59-60°C; $[\alpha]_D^{25}$= -25.9° (C=1.0, MeOH); R_{fA}= 0.64, R_{fD}=0.79; HPLC (H_2O-MeOH 25:75 and 30:70) k'=3.6, k'=6.5 respectively; Anal. ($C_{18}H_{27}NO_5$) C,H,N; ^{1}H NMR ($CDCl_3$/TMS) δ =7.31-7.20 (m, 5H, arom.), 5.13 (bs, 1H, Phe C_αNH), 4.26-4.19 (q, 2H, -O-CH_2-CH_3), 4.07-4.05 (d, 2H, -O-CH_2-CO), 3.91 (bs, 1H, -Phe C_αH), 3.47-3.46 (d, 2H, -CH_2-O-), 2.94-2.87 (m, 2H, Phe $C_\beta H_2$), 1.45 (s, 9H, Boc C(CH_3)$_3$), 1.31-1.26 (t, 3H, -O-CH_2-CH_3); FAB-MS: 338 $(M+H)^+$, 292 $(M+H-C_2H_5OH)^+$, 238 $(M+H-Boc)^+$.

Boc-trans-L-Hyp(Bzl)-OH (8).

To a stirred solution of Boc-trans-4-hydroxy-L-proline (25) 4.0 g (17.3 mmol) in dry THF (100 mL) at 0°C and under N_2 was added sodium hydride (50% oily dispersion) 1.8 g (37.5 mmol) in small portions. The suspension was stirred for 1 h at 0°C and 30 min at room temperature. The reaction mixture was cooled to 0°C and 18-crown-6, 0.045 g (0.17 mmol) was added, followed by benzylbromide 5.1 mL (43.4 mmol). The reaction mixture was stirred under N_2 at 0°C for 1 h and overnight at 50°C, then treated with H_2O (10 mL) and evaporated under reduced pressure. The oily residue was taken up in methanol (70 mL) and 3N NaOH (20 mL) and stirred overnight at 40°C. The solvent was evaporated under reduced pressure and the residue taken up with water (150 mL) and ether (150 mL). The aqueous phase was further extracted with ether (2x100 mL). The combined ethereal washings were extracted with 1N NaOH (50 mL), and the combined aqueous fractions were acidified at 0°C with 2N $KHSO_4$ to pH 2.0-3.0 and extracted with EtOAc (3x100 mL). The organic phase was washed with saturated NaCl solution, dried over $MgSO_4$ and evaporated under reduced pressure to yield pure compound (8) as a pale yellow oil. Yield: 3.9 g (70%); TLC R_{fE}=0.80, R_{fF}=0.46; $[\alpha]_D^{25}$-34.2° (C=1.0, MeOH); Anal. ($C_{17}H_{23}NO_5$) C,H,N.

Boc-trans-L-Hyp(Bzl)-ol (9).

The reduction of Boc-trans-L-4-Hyp(Bzl)-OH (8) to its respective alcohol derivative (9) was carried out essentially as described above for compound 2. Thus, Boc-trans-L-Hyp(Bzl)-OH 3.0 g (9.34 mmol) in 27 mL of dry THF was reacted at -10°C and under N_2, with triethylamine 1.29 mL (9.34 mmol) and ethylchloroformate 0.88 mL (9.34 mmol), and added, after 30 min and filtration of triethylammonium chloride to a solution of sodium borohydride 0.71 g (18.7 mmol) in 7.2 mL of H_2O at 0°C. The reaction mixture was stirred overnight at room temperature then worked up as described above for compound (4). The crude product was further purified by means of LPLC column chromatography on silica gel (230-400 mesh) using a gradient of EtOAc in hexane (0% to 35%) as eluant. The main fractions eluted with 30% EtOAc in hexane, were pooled, the solvent evaporated under reduced pressure to give pure (9) as an oil; Yield: 2.29 (78%); $[\alpha]_D^{25}$-33.3° (C=1.0, MeOH); R_{fA}=0.29; R_{fB}=0.51; R_{fC}=0.76; Anal. ($C_{17}H_{25}NO_4$). C,H,N. ^{1}H NMR ($CDCl_3$/TMS): δ = 7.32 (s, 5H, C_6H_5), 4.50 (s, 2H, -O-CH_2-C_6H_5), 4.07 (bs, 2H, -CH_2-O-), 3.77-3.29 (m, 5H, Hyp C_5H_2, C_4H, C_αH, -CH_2-OH), 2.16-2.02 (m, 2H, Hyp C_3H_2), 1.47 (s, 9H, Boc-C(CH_3)$_3$).

Boc-Trans-L-Hyp(Bzl)ψ(CH_2-O)Gly-OEt (10).

A stirred solution of (9) 2.2 g (7.29 mmol) in dry THF (45 mL) was reacted at 0°C and under N_2 with sodium hydride (50% oily dispersion) 0.38 g (8.02 mmol). The reaction was stirred for 30 min at 0°C and 30 min at room temperature, then cooled to 0°C and 18-crown-6 0.96 g (3.64 mmol) was added followed by a solution of ethyl bromoacetate 0.97 mL (8.75 mmol) in dry THF (5 mL) which was added dropwise during 10 min. The reaction mixture was stirred for 1 h at 0°C and for 2 days at room temperature, then worked up as described above for compound (1). The crude product was further purified by means of LPLC column chromatography on silica gel (230-400 mesh) using a gradient of EtOAc in hexane (0% to 25%) as eluant. The main fractions eluted along with 15% EtOAc in hexane were pooled and evaporated under reduced pressure to give the pure pseudodipeptide unit (10). Yield 1.3 g (42%); $[\alpha]_D^{25}$ -22.1° (C=1.0, MeOH); R_{fA}=0.60, R_{fD}=0.38; HPLC (H_2O-MeOH 30:70) k'=1.20; Anal. ($C_{21}H_{31}NO_6$) C,H,N; ^1H NMR ($CDCl_3$/TMS) δ= 7.32 (s, 5H, C_6H_5), 4.51 (s, 2H, -O-CH_2--C_6H_5), 4.25-4.18 (q, 2H, -O-CH_2-CH_3), 4.08 (s, 2H, -O-CH_2-CO), 4.07-4.06 (bd, 2H, -CH_2-O-), 3.82-3.65 (bs, 2H, Hyp C_5H_2), 3.63-3.36 (bs, 2H, Hyp C_4H, $C_\alpha H$), 2.37-2.05 (m, 2H, Hyp C_3H_2), 1.46 (s, 9H, Boc-C(CH_3)$_3$), 1.31-1.24 (t, 3H, -O-CH_2-CH_3); FAB-MS: 394 [M+H] , 307 [M+H-$CH_2COOC_2H_5$]$^+$, 293 [M+H-Boc]$^+$.

Boc-trans-L-Hyp(Bzl)ψ(CH_2-O)βAla-OEt (11).

A stirred solution of (9) 3.7 g (12 mmol) in dry THF (65 mL) was reacted at 0°C and under N_2 with sodium hydride (50% oily dispersion) 0.75 g (15.6 mmol). The reaction was stirred for 30 min at 0°C and 30 min at room temperature then brought to 0°C and 18-crown-6, 1.58 g (6 mmol) was added followed by a solution of ethyl 3-bromopropionate 2.15 mL (16.8 mmol) in dry THF (5 mL). The reaction mixture was stirred for 2 h at 0°C and for 2 days at room temperature, then worked up in a standard fashion. The crude product was further purified by means of LPLC column chromatography on silica gel (230-400 mesh) using a gradient of EtOAc in hexane (0% to 15%). The main fractions eluted along with 10% EtOAc in hexane were pooled and evaporated under reduced pressure to give the pure pseudodipeptide unit (11). Yield 1.0 g (20%); $[\alpha]_D^{25}$ -36° (C=1.0, MeOH); R_{fA}=0.62, R_{fD}=0.55; HPLC (H_2O-MeOH 30:70 and 35:65), k'=2.40 and k'=2.67 respectively; Anal: ($C_{22}H_{33}NO_6$) C,H,N; ^1H NMR ($CDCl_3$/TMS): δ=7.32 (s, 5H, C_6H_5); 4.50-4.48 (d, 2H, -O-CH_2-C_6H_5), 4.20-4.13 (q, 2H, -O-CH_2-CH_3), 4.09-4.07 (bd, 2H, -CH_2-O-), 3.74-3.68 (t, 2H, -CH_2-CH_2-CO), 3.57-3.44 (m, 4H, Hyp $C_\alpha H$, C_4H, C_5H_2), 2.56-2.50 (t, 2H, -O-CH_2-CH_2-), 2.18-2.08 (m, 2H, Hyp C_3H_2), 1.46 (s, 9H, Boc-C(CH_3)$_3$), 1.30-1.23 (t, 3H, -O-CH_2-CH_3). FAB-MS 407 [M]$^+$, 335 [M+H-$COOC_2H_5$]$^+$, 307 [M+H-Boc]$^+$, 307 [M+H-$CH_2CH_2COOC_2H_5$]$^+$.

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References

1. a) L.L. Iversen, TINS 6, 293 (1983); b) A.J. Harmar, TINS 7, 57 (1984).
2. P.S. Farmer, in "Drug Design" ed. E.J. Ariens, Vol. X, pp. 119-143, Academic Press, New York (1980).
3. A.F. Spatola, Chem. Biochm. Amino Acids & Proteins, 7, 267 (1983).
4. P.W. Schiller, C.F. Yam, and M. Lis, Biochem. 16, 1831 (1977).
5. A.F. Spatola, N.S. Agarwal, A.L. Bettag, J.A. Yankeelov, Jr., C.Y. Bowers and W.W. Vale, Biochem. Biophys. Res. Commun. 97, 1014 (1980).
6. A.F. Spatola, in "Peptides, Structure and Function", Hruby V.J. and Rich D.H. Eds. pp. 341-344 Pierce Chem. Comp. (1983).
7. M. Chorev, R. Shavitz, M. Goodman, S. Minick, R. Guillemin, Science 204, 1210 (1979).
8. M. Chorev, E. Rubini, C. Gilon, U. Wormser, Z. Selinger, J. Med. Chem. 28, 129 (1983).
9. D. Hudson, R. Sharpe, M. Szelke, Int. J. Peptide Protein Res. 15, 122 (1980).
10. R.G. Almquist, W.R. Chao, M.E. Ellis, H.L. Johnson, J. Med. Chem. 23, 1392 (1980).
11. M.T. Cox, D.W. Heaton, J. Horbury, J. Chem. Soc. Chem. Comm. 799 (1980).
12. M.M. Hann, P.G. Sammes, J. Chem. Soc. Chem. Comm. 234, (1980).
13. I. Goldberg, in "Supplement E: The Chemistry of ethers, crown ethers, hydroxyl groups and their sulphur and analogues", S. Patai Ed., John Wiley & Sons, Chapt. 4, pp.175-213, 1980.
14. C.H. Bushweller, M.H. Gianni, ibid 13, Chapt. 5, pp. 215-277, S. Patai, ed.
15. F.J. Tinney, W.H. Roark, E.D. Nicolaides, R.E. Davis, R.E. Voigtman, J.G. Marriot, 9th American Peptide Symp.: Toronto, Abstract WTh-107, pp. 172 (1985).
16. M.A. Ondetti, J. Pluscec, E.R. Weaver, N. Williams, E.F. Sabo, O. Kocy, "Chemistry and Biology of Peptides", Ann Arbor Science Publishers, pp. 525-531 (1972).
17. K. Ishizumi, K. Koga, S. Yamada, Chem. Pharm. Bull., 16, 492 (1968).
18. K. Ramsamy, R.K. Olsen, T. Emery, Synthesis 42, 18 (1982).
19. G.S. Poindexter, A.I. Meyers, Tetrahedron Lett., 40, 3527 (1977).
20. E. Rubini, C. Gilon, D. Levian-Teitelbaum, Z.Selinger, M. Weinstock-Rosin, M. Chorev, in "Peptides 1984" Ragnarsson, U. ed. Almqvist & Wiksell Int. Stockholm, pp. 337-340, 1984.
21. E. Rubini, U. Wormser, D. Levian-Teitelbaum, R. Laufer, C. Gilon, Z. Selinger and M. Chorev, in "Peptides Structure and Function", Deber C.M. Hruby, V.J. and Kopple, K.D. eds. Pierce Chem. Comp. Rockland, Ill. pp. 635-638, 1985.
22. E. Von Arx, M. Foupe and M. Brugger, J. Chromat., 120, 224 (1976).
23. H. Wenker, J. Am. Chem. Soc., 59, 422 (1937).
24. L. Moroder, A. Hallett, O. Keller, G. Wersin, Hoppe-Seyler's, Z. Physiol. Chem., 357, 1651 (1976).
25. G. Perseo, S. Piani, R. De Castiglione, Int. J. Peptide Protein Res., 21, 227 (1983).