AN ALTERNATIVE SYNTHESIS OF $\Psi(CH_2O)$ PSEUDODIPEPTIDES*

Jan HLAVÁČEK and Vladimír KRÁL

Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6

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An alternative route to obtain $\Psi(CH_2O)$ pseudodipeptide unit based on insertion of carbens in hydroxylic bond of alcohols mediated by catalyst rhodium(II) acetate is described. In that way, N-protected derivatives of alaninol, valinol, isoleucinol, leucinol and phenylalaninol were converted, upon a treatment with methyl or ethyl diazoacetate, to corresponding fully protected pseudodipeptides with methyleneoxy isostere bond which can be further used in peptide synthesis.

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. As one of the ways to obtain stable biotransportable peptide drugs, backbone modifications have been introduced into biologically active peptides for several years. Some advantages resulting from these 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 pharmacokinetic properties of the peptides such as: increased oral bioavailability, prolonged duration of action and improved penetration into the CNS.

Among the numerous surrogates of the natural peptide bond CO—NH (refs[1-3]) methyleneoxy modification $\Psi(CH_2O)$ reported in this paper offers a polar, flexible, proteolytically resistant unit with negligible nucleophilicity, with the resistance to oxidation and with a close geometrical resemblance to the amide bond in its extended conformation in unmodified dipeptides. However, this peptide group modification has been used till now rarely since it has only been recently synthesized: by acid hydrolysis of the amide bond in a pyrano lactam derivative[4] or by using the classical method of ether formation by the Williamson's reaction between an amino-alcohol, via an alkoxide, and brominate derivative[5,6]. The last method was modified[7-9] by insertion of a deltalactam intermediate formation step via an intra-

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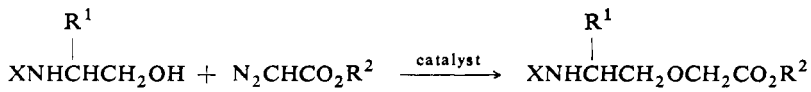
molecular Williamson's reaction which provided higher yields of unprotected pseudodipeptides and was suitable also for hindered amino acids.

In this paper we wish to report a general and easy approach (Scheme 1) to the synthesis of above mentioned methyleneoxy $\Psi(CH_2O)$ isosteric peptide bond.



SCHEME 1

This procedure is based on insertion of carbenes in hydroxylic bond of alcohols mediated by rhodium(II) acetate[10]. The method utilizes easily available starting compounds and gives products in high yields.

In our study, we have chosen methyl or ethyl diazoacetate as the carboxyterminal components generating necessary carbens for the reaction with N-benzyloxycarbonyl derivatives of alaninol*, valinol, isoleucinol, leucinol, phenylalaninol and N-tert-butoxycarbonylleucinol as the aminoterminal alcoholic components to obtain corresponding pseudodipeptides *I* – *VIII*, containing the methyleneoxy bond. This reaction was carried out in the presence of $(AcO)_4Rh_2$ as a catalyst under argon. The fully protected surrogates *I* – *VIII* were ready for use in peptide synthesis via their deprotection either on the amino or carboxy terminus and insertion of the dipeptide unit into the peptide chain.

Compound	X	R*1*	R^2
I	Z	CH_3	CH_3
II	Z	$CH(CH_3)_2$	CH_3
III	Z	$CH(CH_3)C_2H_5$	C_2H_5
IV	Z	$CH_2CH(CH_3)_2$	CH_3
V	Z	$CH_2CH(CH_3)_2$	C_2H_5
VI	Boc	$CH_2CH(CH_3)_2$	C_2H_5
VII	Z	$CH_2C_6H_5$	C_2H_5
VIII	Z	$CH_2C_6H_5$	CH_3

EXPERIMENTAL

Analytical samples were dried over phosphorus pentoxide at room temperature and 150 Pa. Solvents were evaporated on a rotary evaporator (bath temperature 30°C) in vacuo. Thin layer

* The nomenclature and symbols of amino acids obey the published recommendations[11]. The amino acids used in this study are of the L-configuration.

chromatography (TLC) was performed on silica gel coated plates (Silufol, Kavalier, Czechoslovakia) in the following systems: 10% (S1), 20% (S2), 30% (S3), 40% (S4), 50% (S5) of ethyl acetate in petroleum ether, 2-butanol–98% formic acid–water (75 : 13·5 : 11·5) (S6), 2-butanol–aqueous ammonia–water (85 : 7·5 : 7·5) (S7), 1-butanol–acetic acid–water (4 : 1 : 1) (S8), 1-butanol–pyridine–acetic acid–water (15 : 10 : 3 : 6) (S9). Preparative TLC was carried out on the glass plates (20 × 50 cm) covered with a silica (30—60 μ) layer (4 mm). The analytical HPLC was carried out on a 25 × 0·4 cm Vydac column (The Separations Group, Hesperia, U.S.A.), flow rate 60 ml/h, detection at 254 nm, mobile phase a gradient 0—100% of methanol in 0·05M trifluoroacetic acid in 30 min. Preparative HPLC was done on 25 × 0·8 cm Vydac column packed with the same stationary phase, flow rate 180 ml/h, under the same gradient conditions as in the analytical run. Electrophoreses after cleavage the aminoprotecting groups by 33% HBr in acetic acid were performed in a moist chamber on a Whatman 3MM paper (20 V/cm) in a pyridine-acetate buffer pH 5·7 and in a 6% acetic acid pH 2·6 for 45 min. Pseudodipeptide esters were detected with ninhydrin and by the chlorination method. Mass spectrometry with FAB technique was used for determination of M^+ (VG Analytical, England). ^1H NMR spectra were measured on a FT NMR spectrometer Varian XL-200 in DMSO-d_6 with tetramethylsilane as an internal reference. Optical rotations were determined on a Perkin Elmer 141 MCA polarimeter at 20°C.

The N-protected aminoalcohols were prepared by reduction[12] of the corresponding methyl esters of Z-alanine, Z-valine, Z-isoleucine, Z-leucine, Z-phenylalanine and Boc-leucine by $NaBH_4$. The methyl and ethyl α-diazoacetates were prepared from corresponding esters of Gly[13,14].

Z-Ala $\Psi(CH_2O)$Gly-OCH_3 (*I*)

To a stirred solution of Z-alaninol (0·31 g, 1·5 mmol) in dry dichloroethane (30 ml) warmed to 75—80°C under argon, in the presence of rhodium(II) acetate (5 mg), was added methyl diazoacetate (0·2 g, 2 mmol) in dry dichloromethane (10 ml) within 6 h. The second portion of the catalyst (5 mg) was added to the reaction mixture after 3 h of stirring. After cooling the solution was filtered through Celite and solvent evaporated under reduced pressure. The residue was dissolved in ethyl acetate (100 ml) and the solution was washed three times with 50 ml portions of 1M HCl, water, 5% $NaHCO_3$ and water, dried over $MgSO_4$ and evaporated to dryness under reduced pressure. The crude product (0·4 g) was purified by preparative TLC in S2 system to give 0·32 g of *I* as a pale yellow oil. This product was further purified by preparative HPLC with yield 0·28 g (66%). $E^{Gly}_{2.6}$ 1·27, $E^{His}_{2.6}$ 0·71, $E^{His}_{5.7}$ 0·92. R_F 0·30 (S2), 0·71 (S6), 0·41 (S7), 0·59 (S8), 0·61 (S9). Gradient HPLC retention time was 13·31 min. $[\alpha]_D^{20}$ −14·8° (*c* 2·0, MeOH). For $C_{14}H_{19}NO_5$ (281·3) calculated: 59·78% C, 6·81% H, 4·98% N; found: 59·64% C, 7·02% H, 5·06% N. Mass spectrum (FAB), *m/z*: 282 ($M^+ + 1$). ^1H NMR spectrum: 7·35 s (5 H, phenyl): 7·10 d (1 H, NH): 5·02 s (2 H, CH_2Ph); 4·11 s (2 H, CH_2Gly); 3·93 m (1 H, CHAla); 3·62 s (3 H, CH_3O); 3·33 d (2 H, CH_2O); 0·82 d (3 H, CH_3Ala).

Z-Val $\Psi(CH_2O)$Gly-OCH_3 (*II*)

To a stirred solution of Z-valinol (0·38 g, 1·6 mmol) in dry dichloroethane (25 ml) at 75—80°C under argon was added methyl diazoacetate (0·22 g, 2·2 mmol) within 8 h in the presence of rhodium (II) acetate (5 mg). The same portion of the catalyst was added after 3 h of stirring of the mixture which was after cooling worked up as described for compound *I*. The crude product was purified by preparative TLC using the solvent system S4 with yield of 0·48 g of pale yellow oil which was further purified by means of HPLC to give the pure compound *II* in a yield 0·39 g (79%). $E^{Gly}_{2.6}$ 1·21, $E^{His}_{2.6}$ 0·67, $E^{His}_{5.7}$ 0·88. R_F 0·63 (S4), 0·82 (S5), 0·76 (S6), 0·49 (S7), 0·69

(S8), 0·70 (S9). Gradient HPLC retention time was 14·62 min. $[\alpha]_D^{20}$ −18·3° (*c* 2·0, MeOH). For $C_{16}H_{23}NO_5$ (309·4) calculated: 62·11% C, 7·49% H, 4·53% N; found: 61·84% C, 7·53% H, 4·69% N. Mass spectrum (FAB), *m/z*: 310 ($M^+ + 1$). ^{1}H NMR spectrum: 7·35 s (5 H, phenyl); 6·93 d (1 H, NH); 5·03 s (2 H, CH_2Ph); 3·98 s (2 H, CH_2Gly); 3·69 s (3 H, CH_3O); 3·54 m (1 H, αCHVal); 3·31 d (2 H, CH_2O), 1·81 m (βCHVal), 0·82 t (6 H, 2 × γCH_3).

Z-Ile Ψ(CH_2O)Gly-OC_2H_5 (*III*)

To a stirred solution of Z-isoleucinol (0·25 g, 1 mmol) in dry dichloroethane (20 ml), at 75—80°C under argon was added ethyl diazoacetate (0·17 g, 1·5 mmol) in dichloroethane (15 ml) within 8 h. Rhodium(II) acetate (8 mg) was added to the reaction in two partions — at the beginning and after 3 h. The reaction mixture was worked up as described in the preparation of compound *I* and the residue was chromatographed using preparative TLC in S4 system with yield 0·32 g of pale yellow oil which was further purified by HPLC to give 0·27 g (80%) of pure pseudodipeptide *III*. $E_{2.6}^{Gly}$ 1·14, $E_{2.6}^{His}$ 0·64, $E_{5.7}^{His}$ 0·72. R_F 0·43 (S4), 0·78 (S6), 0·69 (S7) 0·77 (S8), 0·76 (S9). Gradient HPLC retention time was 16·15 min. $[\alpha]_D^{20}$ −14·6° (*c* 1·2, MeOH). For $C_{18}H_{27}NO_5$ (337·4) calculated: 64·08% C, 8·07% H, 4·15% N; found: 63·79% C, 8·16% H, 4·39% N. Mass spectrum (FAB), *m/z*: 338 ($M^+ + 1$). ^{1}H NMR spectrum: 7·53 d (1 H, NH); 7·35 s (5 H, phenyl); 5·03 s (2 H, CH_2Ph); 4·19 q (2 H, CH_2 ethyl ester); 4·14 s (2 H, CH_2Gly); 4·10 m (1 H, αCHIle); 3·33 d (2 H, CH_2O); 1·52 m (1 H, βCHIle); 1·38 m (2 H, γCH_2Ile); 1·23—1·16 t (3 H, CH_3 ethyl ester); 0·82 q (6 H, γ and δCH_3Ile).

Z-Leu Ψ(CH_2O)Gly-OCH_3 (*IV*)

To a stirred solution of Z-leucinol (0·4 g, 1·6 mmol) in dry dichloroethane (30 ml) at 75—80°C under argon was added methyl diazoacetate (0·24 g, 2·4 mmol) in dichloroethane (20 ml) within 8 h. Rhodium(II) acetate (10 mg) was added to the reaction mixture in two portions — at the beginning and after 3 h of the reaction. The product was worked up as described for compound *I* and was purified by preparative TLC and HPLC with overall yield 0·43 g (83%) of pure compound *IV*. $E_{2.6}^{Gly}$ 1·14, $E_{2.6}^{His}$ 0·64, $E_{5.7}^{His}$ 0·81. R_F 0·58 (S3), 0·77 (S6), 0·74 (S7), 0·86 (S8), 0·78 (S9). Gradient HPLC retention time was 14·81 min. $[\alpha]_D^{20}$ −21·8° (*c* 0·5, MeOH). For $C_{17}H_{25}NO_5$ (323·4) calculated: 63·14% C, 7·79% H, 4·33% N; found: 62·87% C, 7·73% H, 4·51% N. Mass spectrum (FAB), *m/z*: 324 ($M^+ + 1$). ^{1}H NMR spectrum: 7·34 s (5 H, phenyl); 7·10 d (1 H, NH); 5·01 s (2 H, CH_2Ph); 4·11 s (2 H, CH_2Gly); 3·98 m (1 H, αCHLeu); 3·64 s (3 H, CH_3O); 3·33 d (2 H, CH_2O); 1·6 low m (1 H, γCHLeu); 1·26 low t (2 H, βCH_2Leu); 0·85 q (6 H, 2 × δCH_3Leu).

Z-Leu Ψ(CH_2O)Gly-OC_2H_5 (*V*)

To a stirred solution of Z-leucinol (0·25 g, 1 mmol) in dry dichloroethane (10 ml) was under the same conditions as described for compound *I* added ethyl diazoacetate (0·17 g, 1·5 mmol) in the presence of rhodium(II) acetate (8 mg). The reaction mixture was worked up in the usual manner and a crude oily product *V* was purified by preparative TLC and HPLC to give pure *V* (yield 0·25 g, 74%). $E_{2.6}^{Gly}$ 1·11, $E_{2.6}^{His}$ 0·62, $E_{5.7}^{His}$ 0·73. R_F 0·67 (S3), 0·79 (S6), 0·73 (S7), 0·72 (S8), 0·76 (S9). Gradient HPLC retention time was 15·2 min. $[\alpha]_D^{20}$ −23·9° (*c* 0·4, MeOH). For $C_{18}H_{27}NO_5$ (337·4) calculated: 64·08% C, 8·07% H, 4·15% N; found: 63·82% C, 7·96% H, 4·38% N. Mass spectrum (FAB), *m/z*: 338 ($M^+ + 1$). ^{1}H NMR spectrum: 7·33 s (5 H, phenyl); 7·12 d (1 H, NH); 5·03 s (2 H, CH_2Ph); 4·27 q (2 H, CH_2 ethyl ester); 4·09 s (2 H, CH_2Gly); 3·96 m (1 H, αCHLeu); 3·36 d (2 H, CH_2O); 1·57 low m (1 H, γCHLeu); 1·36 low t (2 H, βCH_2Leu); 1·19 t (3 H, CH_3 ethyl ester); 0·87 q (6 H, 2 × δCH_3Leu).

Boc-Leu $\Psi(CH_2O)$Gly-OC_2H_5 (*VI*)

The same reaction and purification conditions as described for compound *I* were used in coupling Boc-leucinol (0·31 g, 1·5 mmol) with ethyl diazoacetate (0·26 g, 2·3 mmol). The yield of pure pseudodipeptide *VI* was 0·38 g (83%). $E_{2,6}^{Gly}$ 1·23, $E_{2,6}^{His}$ 0·68, $E_{5,7}^{His}$ 0·86. R_F 0·64 (S3), 0·79 (S6), 0·73 (S7), 0·72 (S8), 0·76 (S9). Gradient HPLC retention time was 16·45 min. $[\alpha]_D^{20}$ −29·4° (*c* 1·2, MeOH). For $C_{15}H_{29}NO_5$ (303·4) calculated: 59·38% C, 9·63% H, 4·62% N; found: 59·12% C, 9·68% H, 4·79% N. Mass spectrum (FAB), *m/z*: 304 ($M^+ + 1$). ^{1}H NMR spectrum: 7·12 d (1 H, NH); 4·22 q (2 H, CH_2 ethyl ester); 4·06 s (2 H, CH_2Gly); 3·97 m (αCHLeu); 3·32 d (2 H, CH_2O); 1·62 low m (1 H, γCHLeu); 1·48 s (9 H, tert-butyl); 1·29 low t (2 H, βCH_2Leu); 1·17 t (3 H, CH_3 ethyl ester); 0·83 q (6 H, 2 × δCH_3Leu).

Z-Phe $\Psi(CH_2O)$Gly-OC_2H_5 (*VII*)

Using the reaction and purification conditions described for the synthesis of the pseudodipeptide *I*, Z-phenylalaninol (0·28 g, 1 mmol) was coupled with ethyl diazoacetate (0·17 g, 1·5 mmol) in the presence of rhodium(II) acetate (8 mg) to obtain 0·30 g (81%) of the pure compound *VII* as a pale yellow oil. $E_{2,6}^{Gly}$ 0·88, $E_{2,6}^{His}$ 0·47, $E_{5,7}^{His}$ 0·67. R_F 0·36 (S1), 0·81 (S6), 0·80 (S7), 0·91 (S8), 0·82 (S9). Gradient HPLC retention time was 21·4 min. $[\alpha]_D^{20}$ −18·8° (*c* 1·05, MeOH). For C_{21}. $.H_{25}NO_5$ (371·4) calculated: 67·91% C, 6·78% H, 3·77% N; found: 67·68% C, 6·75% H, 3·92% N. Mass spectrum (FAB), *m/z*: 372 ($M^+ + 1$). ^{1}H NMR spectrum: 7·73 d (1 H, NH); 7·32—7·25 m (10 H, 2 × phenyl); 6·42 s (2 H, βCH_2Phe); 5·03 s (2 H, CH_2Ph); 4·40 m (αCHPhe); 4·16 q (2 H, CH_2 ethyl ester); 4·13 s (2 H, CH_2Gly); 3·33 d (2 H, CH_2O); 1·31–1·26 t (3 H, CH_3 ethyl ester).

Z-Phe $\Psi(CH_2O)$Gly-OCH_3 (*VIII*)

Z-phenylalaninol (0·28 g, 1 mmol) was coupled with methyl diazoacetate (0·15 g, 1·5 mmol) in the presence of rhodium(II) acetate under conditions described in the preparation of compound *I*. The same procedure was also used for working up the reaction mixture and purification of crude product by preparative TLC and HPLC to give 0·27 g (76%) of the pure pseudodipeptide *VIII*. $E_{2,6}^{Gly}$ 0·94, $E_{2,6}^{His}$ 0·51, $E_{5,7}^{His}$ 0·69. R_F 0·31 (S1), 0·78 (S6), 0·81 (S7), 0·89 (S8), 0·87 (S9). Gradient HPLC retention time was 19·7 min. $[\alpha]_D^{20}$ −17·4° (*c* 2·0, MeOH). For $C_{20}H_{23}NO_5$ (357·4) calculated: 67·21% C, 6·49% H, 3·92% N; found: 67·54% C, 6·40% H, 4·19% N. Mass spectrum (FAB), *m/z*: 358 ($M^+ + 1$). ^{1}H NMR spectrum: 7·72 d (1 H NH); 7·30—7·24 m (10 H, 2 × phenyl); 6·45 s (2 H, βCH_2Phe); 5·01 s (2 H, CH_2Ph); 4·42 m (αCHPhe); 4·14 s (2 H, CH_2Gly); 3·62 s (3 H, CH_2O); 3·35 d, 2 H, CH_2O).

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