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Preparation of Protected α-Methoxyglycine and Its Incorporation into Peptide Synthesis

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A protected α-methoxyglycine Cbz-DL-Gly(OMe)-OMe (**1**) was prepared from the N-chloro derivative of Cbz-Gly-OMe. Catalytic hydrogenolysis of **1** in the presence of a mixed anhydride prepared from a Boc-amino acid and $ClCO_2Bu^i$ gave a diastereomeric protected dipeptide containing the Gly(OMe) residue. The dermorphin analogs L-Tyr-D/L-Gly(OMe)-L-Phe-Gly-NH_2 were synthesized.

Base catalyzed dehydrochlorination of N-chloro derivative of an N-protected α-amino acid affords an α-imino carboxylic acid derivative, which furnishes an α-methoxy-α-amino acid derivative by the addition of a methanol molecule to the N=C double bond.[1] However, acid or base treatment of the methoxy compound easily yields an α,β-dehydro amino acid derivative by losing a methanol molecule. On the other hand, the N-chlorination–dehydrochlorination sequence was applied to penicillins to prepare 6α-methoxypenicillins,[2] which did not undergo demethoxylation because the corresponding α,β-dehydro compounds would contain a highly strained endocyclic double bond in the β-lactam ring. In the case of glycine possessing no atoms at β-position, the corresponding α-methoxy derivative cannot be transformed to an α,β-unsaturated compound and is also expected to be stable. Since synthesis of peptides containing the α-methoxyglycine residue has not been reported, it is worthwhile to establish a general method for the synthesis of α-methoxyglycine-containing peptides to study their properties. In this communication preparation of a protected α-methoxyglycine and its use for peptide synthesis is described.

The N-chloro derivative Cbz-$NClCH_2CO_2Me$ (10 mmol), obtained from Cbz-Gly-OMe and Bu^tOCl, was treated with NaOMe (15 mmol) in MeOH (25 ml) at

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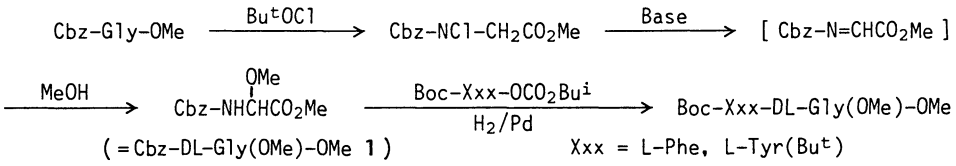
-78 °C for 3 h to give a racemic α-methoxyglycine derivative Cbz-DL-Gly(OMe)-OMe (**1**) in 72% yield; mp 73-74.5 °C (from MeOH); ^{1}H NMR δ^{CDCl_3} 3.44 (s, OMe), 3.79 (s, CO_2Me), 5.16 (s, CH_2O), 5.34 (d, J=9 Hz, $C^{\alpha}H$), 5.9 (br d, NH), 7.35 (s, C_6H_5); ^{13}C NMR δ^{CDCl_3} 52.9 (Me ester), 56.4 (Me ether), 67.4 (CH_2O), 80.6 (C^{α}), 128.2 (C^{o} or C^{m}), 128.4 (C^{p}), 128.6 (C^{o} or C^{m}), 135.7 (C^{ipso}), 155.7 (OCONH), 168.0 (CO_2). Alkaline hydrolysis of the methyl ester **1** afforded Cbz-DL-Gly(OMe)-OH [mp 90-90.5 °C; yield 95%; ^{1}H NMR δ^{CDCl_3} 3.47 (s, OMe), 5.16 (s, CH_2O), 5.34 (d, J=9 Hz, $C^{\alpha}H$), 5.9 (br d, NH), 7.34 (s, C_6H_5), 8.3 (br s, CO_2H)], which was stable enough to be subjected to coupling reaction with an amino component. Deprotection of the Cbz group of **1** by catalytic hydrogenolysis, however, yielded unstable H-Gly(OMe)-OMe which could not be isolated. As a model reaction coupling of H-Gly(OMe)-OMe with Boc-L-Phe-OH was attempted under various conditions. Without separating the product of hydrogenolysis of **1**, the reaction mixture was subjected to the coupling reaction using DCC-HOBt, which did not give the desired dipeptide but resulted in the isolation of Boc-L-Phe-Gly-OMe. Since Boc-Gly(OMe)-OMe was stable under the condition of catalytic hydrogenolysis, the dipeptide lacking α-methoxy group was assumed to be obtained by the coupling of Boc-L-Phe-OH with H-Gly-OMe presumably formed by the loss of MeOH from H-Gly(OMe)-OMe followed by hydrogenation of the imino double bond. Attempted coupling reaction of H-DL-Gly(OMe)-OMe generated in situ with Boc-L-Phe-ONSu was also unsuccessful affording complex mixture containing Boc-L-Phe-Gly-OMe. Catalytic hydrogenolysis of **1** over Pd-C in THF at -15 °C in the presence of a mixed anhydride prepared from Boc-L-Phe-OH and $ClCO_2Bu^{i}$, however, was found to give the desired diastereomeric dipeptide Boc-L-Phe-DL-Gly(OMe)-OMe in 64% yield. By this method the Gly(OMe) residue can be incorporated as a building block in peptides. For the purpose of examining general applicability of the mixed anhydride–hydrogenolysis procedure and studying properties of Gly(OMe) residue-containing peptides especially their stability under deprotection conditions, synthesis of analogs of dermorphin N-terminal tetrapeptide L-Tyr-D-Ala-L-Phe-Gly-NH_2[3] possessing D/L-Gly(OMe) in place of D-Ala has been undertaken.



Coupling reaction of Boc-L-Tyr(Bu^{t})-OCO_2Bu^{i} (2 mmol) with H-DL-Gly(OMe)-OMe formed in situ from **1** (1.34 mmol) in THF (7 ml) at -15 °C for 10 h afforded diastereomeric dipeptides Boc-L-Tyr(Bu^{t})-DL-Gly(OMe)-OMe

(79%). The product without separation of each diastereomer was saponified to give Boc-L-Tyr(Bu^t)-DL-Gly(OMe)-OH, which was subjected to the coupling reaction with H-L-Phe-Gly-NH_2 using DCC-HOBt. The product showed two spots on silica gel TLC (R_f 0.41 and 0.35; $CHCl_3$: MeOH : AcOH = 92 : 5 : 3) and column chromatographic separation on silica gel using $CHCl_3$-MeOH as eluent enabled isolation of the two components; higher R_f compound, mp 164-165 °C, yield 28% and lower R_f compound, mp 109-110 °C, yield 30%. Both of the compounds showed pseudomolecular peaks at *m/z* 628 (M + H) and 650 (M + Na) on FAB-mass spectra in agreement with the protected tetrapeptide structures. Their 400 MHz ^1H NMR spectra taken in DMSO-d_6 solution were similar to each other confirming their diastereomeric relationship as summarized in Table 1. Remarkable difference between their spectra was that the methoxy signal of the Gly(OMe) residue of the lower R_f diastereomer was observed at much higher field (δ 2.97) than that of the higher R_f

Table 1. 400 MHz ^1H NMR Spectral Data of Protected and Unprotected Analogs of Dermorphin N-Terminal Tetrapeptides Determined in DMSO-d_6 Solution

Chemical shifts (δ/PPM) and multiplicities are given with coupling constants (J/Hz) in parentheses.

	Xxx-Yyy-L-Phe-Gly-NH_2			
Xxx Yyy	Boc-L-Tyr(OBu^t) D-Gly(OMe)	Boc-L-Tyr(OBu^t) L-Gly(OMe)	HCl·L-Tyr D-Gly(OMe)	HCl·L-Tyr L-Gly(OMe)
Boc	1.25 s	1.26 s		
Tyr				
NH	6.98 d (8.8)	6.98 d (8.8)	7.95 br	8.15 br
α	4.25 m	4.17 m	3.99 br t (≈7)	4.02 dd (7.8, 4.4)
β	2.66 dd (13.2, 11.2)	2.66 dd (13.7, 11.2)	2.83 dd (≈14, ≈9)	2.80 dd (14.2, 7.8)
	2.92 dd (13.2, 3.9)	2.88 dd (13.7, ≈4)	2.98 dd (14.7, 5.9)	2.96 dd (14.2, 4.4)
arom	6.84 d (8.1)	6.84 d (8.5)	6.70 d (8.3)	6.69 d (8.3)
	7.19 d (8.1)	7.16 d (8.5)	7.08 d (8.3)	7.05 d (8.3)
OBu^t	1.28 s	1.28 s		
OH			9.38 s	9.39 s
Gly(OMe)				
NH	8.54 d (8.8)	8.57 d (9.2)	9.16 d (8.3)	9.42 d (8.8)
α	5.39 d (8.8)	5.18 d (9.2)	5.41 d (8.3)	5.19 d (8.8)
OMe	2.97 s	3.22 s	2.92 s	3.26 s
Phe				
NH	8.36 d (8.3)	8.24 d (7.8)	8.52 d (8.3)	8.36 d (8.3)
α	4.56 m	4.56 m	4.56 m	4.60 m
β	2.88 dd (13.9, 9.3)	2.90 dd (13.7, 9.3)	2.89 dd (≈13, ≈9)	2.91 dd (13.7, 9.3)
	3.10 dd (13.9, 4.7)	3.09 dd (13.7, 4.6)	3.09 dd (13.3, 4.5)	3.16 dd (13.7, 4.9)
arom-p	≈7.2 m	≈7.15 m	≈7.2 m	7.17 m
-o,m	7.26 m	7.22 m	7.26 m	7.25 m
Gly				
NH	8.31 dd (5.9, 5.7)	8.30 t (4.9)	8.38 br t (≈6)	8.40 t (≈5.7)
α	3.60 dd (16.6, 5.7)	3.60 dd (16.6, 4.9)	3.58 dd (11.6, 5.9)	3.62 dd (16.6, 5.9)
	3.68 dd (16.6, 5.9)	3.71 dd (16.6, 4.9)	3.70 dd (16.6, 5.9)	3.72 dd (16.6, 5.4)
NH_2	7.07 br s	≈7.15 br s	7.09 br s	7.10 br s
	7.15 br s	≈7.2 br s	≈7.2 br s	7.24 br s

diastereomer (δ 3.22). It is known that the methyl signal of D-Ala residue in dermorphin-related peptides is more shielded than the corresponding biologically inactive L-Ala analogs.[4] Consequently, D-configuration has been assigned tentatively to the Gly(OMe) residue of the lower R_f diastereomer, namely Boc-L-Tyr(Bu^t)-D-Gly(OMe)-L-Phe-Gly-NH_2, and all L-configuration to the higher R_f diastereomer. Removal of the protecting groups of the Tyr residue in each of these tetrapeptides (0.1 mmol) was undertaken using 4M HCl/dioxane (0.14 mmol) at -10 °C for 30 min. After the addition of cold ether the precipitated HCl salt was filtered and was purified by silica gel and Sephadex LH-20 column chromatography affording the deprotected tetrapeptide; HCl·L-Tyr-D-Gly(OMe)-L-Phe-Gly-NH_2, mp 146-148 °C, yield 52%; HCl·L-Tyr-L-Gly(OMe)-L-Phe-Gly-NH_2, mp 182-184 °C, yield 56%. The ^1H NMR spectra given also in Table 1 are consistent with the proposed structures. Methoxy signal of the D-Gly(OMe) residue in the deprotected tetrapeptide ($\delta^{DMSO\text{-}d_6}$ 2.92) resonated also at higher field than the methoxy signal of all-L diasteromer ($\delta^{DMSO\text{-}d_6}$ 3.26), suggesting that these Gly(OMe)-containing analogs adopt similar conformation to the corresponding D- and L-Ala compounds.

The Gly(OMe)-containing analogs of N-terminal tetrapeptides of dermorphin have been synthesized successfully demonstrating that the novel Gly(OMe) residue can be incorporated in the peptides. The synthetic route, however, included separation of diastereomers since racemic Gly(OMe) derivative was used as a starting material. Optical resolution of Cbz-DL-Gly(OMe)-OH is in progress.

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