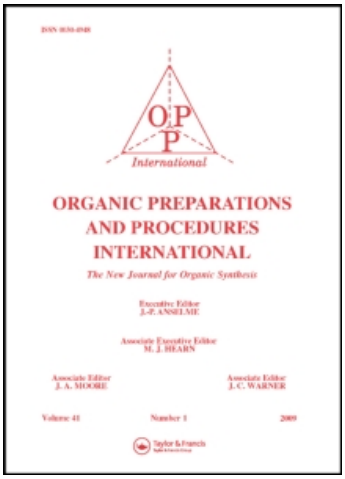


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# THE SUCCESSFUL SYNTHESIS OF GLYCYL-GLYCINE PEPTIDE BONDS VIA THE EXCESS MIXED ANHYDRIDE METHOD

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# THE SUCCESSFUL SYNTHESIS OF GLYCYL-GLYCINE PEPTIDE BONDS VIA THE EXCESS MIXED ANHYDRIDE METHOD

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Several literature references[1,2,3,4] suggest the possible side reaction of double acylation of amino groups or urethane acylation while using mixed anhydrides to form peptide bonds. The double acylation is assumed to be more pronounced during the formation of glycyl-glycine peptide bonds and the syntheses of such sequences via the mixed anhydride activations are therefore usually avoided. Merrifield et al.[2] recently reported the incorporation of multiple amino acid residues in a peptide during the reaction of BPOC-amino acid mixed anhydride. This observed polymerization may be the result of the partial removal of the protective biphenyloxycarbonyl (BPOC) group by tertiary base salts of strong mineral acids such as N-methyl morpholine HCl; examples of such deblockings are reported in the literature.[5] The same authors stated that absolutely no acylation of protected amino groups on the resin occurred in standard solid phase procedure or on treatment with mixed anhydrides. This may be due to the inability of $Et_3N \cdot HCl$ to deprotect the amino group while it is on the resin; consequently, there is no acylation. The polymerization of the BPOC-glycine mixed anhydride in solution thus appears to be the result of the very acid labile nature of the BPOC group rather than the tendency of mixed anhydrides to cause diacylation. The mixed anhydrides derived from ethyl chloroformate have been reported to

lead to the formation of considerable amounts of diacylated side products.[1,2] Thus, Kopple and Renick[1] reported isolation of a 30% diacylated side product during synthesis of Z-gly-gly-O-Et via the mixed anhydride derived from ethyl chloroformate with only a 1:1 ratio of the reactants. In contrast, our results suggest that the use of isobutyl chloroformate does not lead to detectable levels of diacylated side-products even though 1.6 equivalent of the mixed anhydride was used. The use of mixed anhydride forming reagents with comparable steric hindrance (e.g. pivalic chloride) therefore seemed preferable in case of peptide couplings which are more prone to form diacylated side products.

Schellenberg and Ullrich[6] reported formation of diacylamides during the synthesis of Z-gly-glu(OEt)-OEt while using Z-gly mixed anhydride derived from isobutyl chloroformate. Due to the hydrogen bond formation between the γ-carbonyl of glutamic acid and the urethane -NH, the nitrogen may become more susceptible to the attack by vigorous acylating agents such as mixed anhydrides. This may also explain the 17% side reaction of the diethyl glutamate as opposed to only a small amount of diacylation observed with dibenzyl glutamate which would sterically restrict the extent of the diacylation. Schellenberg and Ullrich state that neither the increased ratio (2:1) of Z-glycine mixed anhydride over the amino component nor the longer reaction times afforded the expected higher yields of the diacylated side products. The diacylation may be occurring mainly during the associative state between the mixed anhydride and the glutamic acid diester and the extent of the diacylation therefore may not depend on the ratio of the mixed anhydride used. The observed diacylation may be the property of glutamic acid diesters when reacted with the mixed anhydrides.

As a severe test for the supposed tendency of the mixed anhydrides to cause diacylation of the amino group, we synthesized the known compounds benzyloxycarbonyl (Z)-gly-gly-OEt and Z-gly-gly-gly-OEt via the excess mixed anhydride method of peptide synthesis.[7,8] Glycine is less sterically hindered and should facilitate any tendency to undergo double acylation. Furthermore, the excess mixed anhydride method, by providing an excess acylating agent, would tend to augment such a diacylation side reaction.

The syntheses of both peptides, however, resulted in surprisingly pure compounds which according to detailed analyses contain no detectable amounts of side products. The experimental procedures we followed should not logically remove any diacylated or urethane acylated compounds unless such products are hydrolyzed by $KHCO_3/H_2O$ under conditions employed below. In our opinion this is not very likely because diacylated products reported by Kopple and Renick[1] and Schellenberg and Ullrich[6] were isolated after bicarbonate washes at room temperature. We must conclude that no such side reactions occurred at a level that could be detected by analysis including several chromatographic systems.



## EXPERIMENTAL

All mps are uncorrected. NMR spectra were measured on a Varian Model T-60 NMR Spectrometer in $d_6$-DMSO using TMS as internal standard. Elemental analyses were performed on a Perkin-Elmer 240 Analyzer. Thin layer chromatographic (TLC) results were obtained on Silical Gel 60 F-254 (E. Merck, Darmstadt, Germany). Solvent systems used were; A. 93:7:10 THF:cyclohexane:$H_2O$ B. 75:24:1 ether:MeOH:$H_2O$ C. 75:24:1 $CHCl_3$:MeOH:$H_2O$ D. 75:24:1 $CHCl_3$:BuOH:$H_2O$ E. 90:10 $CHCl_3$:HOAc. High Pressure Liquid Chromatography (HPLC) was performed by placing a 100 mcg sample of peptide on a 2ft. x 4 mm column with $C_{18}$ Carasil reverse phase packing (Waters Assoc.) and eluting at 300 psi and a flow rate of 100 ml/hr with a solvent gradient consisting of 15% $CH_3CN$ in 0.05M, pH 8.5 sodium borate buffer and 1% $CH_3CN$. The eluent was monitored at 254 nm.

Z-gly-gly-OEt. - To 320 mM (66.97 g.) Z-gly-OH in 200 ml DMF at -15° was added 35.76 ml (319.97 mM) N-methyl morpholine (NMM) and 39.73 ml (319.97 mM) isobutyl chloroformate (IBCF). After stirring at -15° for 5

minutes, a chilled solution of H-gly-OEt (prepared by neutralization of 27.916 g. (200 mM) H-gly-OEt·HCl in 150 ml DMF with 22.55 ml (200.2 mM) NMM) was added. The mixture was stirred for 3 hr. at -15° and then stored at -15° for 16 hr. A saturated aqueous solution of $KHCO_3$ at 0° was added to maintain pH 8 for 30 min. at 0°. One liter of 50% saturated $NaCl/H_2O$ at 0° was added. The precipitate was filtered and washed well with water. The product was then dissolved in EtOAc, treated with decolorizing carbon and the solution was evaporated on a rotary evaporator to a solid. Trituration with petroleum ether afforded 45.319 g. (77%) of a white, crystalline solid, mp. 80-82°.

Anal. Calcd. for $C_{14}H_{18}N_2O_5$: C, 57.14; H, 6.06; N, 9.52

Found: C, 57.41; H, 6.05; N, 9.20

$^1$H-NMR(DMSO$d_6$): $\delta$ = 1.2 (t,3H), 3.9 (m, 4H), 4.15 (m, 2H), 5.1 (s, 2H), 6.05 (m, 1H), 7.2 (m, 1H), 7.4 (s, 5H). TLC, A. $R_f$= 0.73 B. $R_f$= 0.79 C. $R_f$= 0.87 D. $R_f$= 0.78 E. $R_f$= 0.75.

Z-gly-gly-gly-OEt. - 10 mM (2.9431 g.) of the above Z-gly-gly-OEt was hydrogenolyzed in 50 ml MeOH plus 10 ml 1N HCl with $H_2\uparrow$ and 5% Pd/C (wetted with AcOH) for 4 hr. at 23°. After filtration and evaporation of the filtrate to an oil, 10 ml of DMF was added. The dipeptide ester hydrochloride was neutralized with 1.12 ml (10 mM) NMM and the solution was cooled to -15°. In a separate flask, 16 mM (3.3483 g.) Z-gly-OH dissolved in 30 ml DMF was cooled to -15°. 1.8 ml (16 mM) NMM and 2.0 ml (16 mM) IBCF were added and the solution was stirred 5 min. at -15°. The chilled dipeptide solution above was added to the mixed anhydride solution at -15°. The reaction mixture was stirred 1 hr. and then stored 18 hr. at -15°. The usual treatment with $KHCO_3$ and 100 ml of 50% saturated $NaCl/H_2O$ followed by filtration and washing with water yielded 3.1114 g. (88%) of a white, amorphous solid, mp. 150-153°.

Anal. Calcd. for $C_{16}H_{21}N_3O_6$: C, 54.70; H, 6.02; N, 11.96

Found: C, 54.73; H, 6.06; N, 11.87

$^1$H-NMR(DMSOd$_6$): $\delta$ = 1.2 (t, 3H), 3.8 (m, 6H), 4.1 (m, 2H), 5.1 (s, 2H), 7.4 (s, 5H), 8.2 (m, 2H). TLC, A. $R_f$= 0.55 E. $R_f$= 0.22. HPLC, a single symmetrical peak with a retention time of 0.24 hours.

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