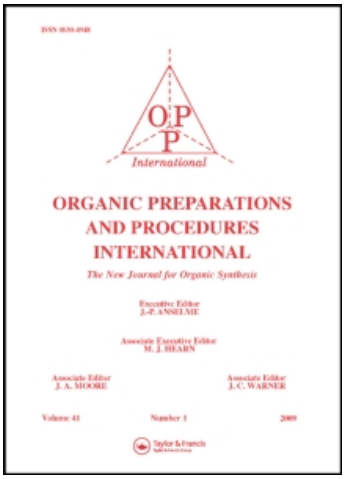

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A SIMPLE PREPARATION OF MONO-*tert*-BUTYL BENZYLOXYCARBONYL-L-GLUTAMATES

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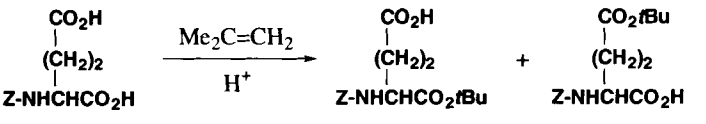
The title compounds Z-Glu(O*t*Bu) and Z-Glu-O*t*Bu[1] serve as valuable intermediates in Schwyzer's strategy of chain assembly of α- and γ-glutamyl peptides respectively, using the benzyloxycarbonyl group for temporary protection and *tert*-butyl type groups for permanent protection of reactive functions.[2,3] Moreover, Z-Glu-O*t*Bu is a key substrate in the synthesis of folate and antifolate poly-γ-glutamic acids of various chain length.[4,5] Currently, mono-*tert*-butyl *N*-protected glutamates are obtained in multistep procedures[6,7] except for one[7] in which free glutamic acid is esterified with isobutene, followed by *N*-acylation and resolution of the resulting mixture by crystallization. The method produces predominantly the γ- rather than the α-*tert*-butyl ester. However from the perspective of antifolate poly-γ-glutamyl conjugates,[4,5] the α-ester is of greater interest. In order to obtain the latter as the major product, we reversed the reaction sequence, *i. e.*, Z-Glu, rather than the free glutamic acid, was esterified with isobutene. As expected, the α-ester was formed as the main product. Herein we report on the preparation of Z-Glu-O*t*Bu and Z-Glu(O*t*Bu), based on the action of liquid isobutene on Z-Glu and resolution of the resulting species.

Z-Glu(O*t*Bu)-O*t*Bu was first removed from the reaction mixture after basification, by extraction with ether. The unreacted Z-Glu and both monoesters were then extracted from the aqueous solution after acidification to pH 3 and Z-Glu was reextracted with 0.01M K_2CO_3; finally the monoesters were separated by fractional crystallization exploiting the difference in solubility of dicyclohexylamine (DCHA) salts of isomeric acids as in the case of mono-*tert*-butyl 9-fluorenylmethoxycarbonylglutamates.[7] The process described is very simple and provides pure (HPLC) Z-Glu-O*t*Bu·DCHA and Z-Glu(O*t*Bu) in 33% and 22% yield respectively (total yield 55% for both



Z-Glu *Z-Glu-OtBu* *Z-Glu(OtBu)*

esters). Z-Glu-O*t*Bu·DCHA may be stored for about half year without any change in mp. and chromatographic behavior. After this time, unidentified impurities were detected by TLC. Z-Glu-O*t*Bu itself is much more stable; after three years, no degradation was noted. On the other hand, Z-Glu(O*t*Bu) is less stable; after three months of standing, the presence of Z-Glu was detected.

EXPERIMENTAL SECTION

Purified solvents (Polskie Odczynniki Chemiczne) were stored over drying agents. Organic solutions were dried over anhydrous Na_2SO_4 and solvents were removed *in vacuo* on a rotatory evaporator at bath temperatures not exceeding 30° unless otherwise indicated. The esterification and workup were monitored and the homogeneity of esters checked on silica gel plates (DC Alufolien Kieselgel 60 No 5553 Merck) in system chloroform-methanol-acetic acid (95:5:3). Spots were visualized with chlorine-KI-tolidine reagent and bromocresol green. Mps. were determined on a Boetius heating block and are uncorrected. HPLC analyses were performed on a Beckman "System Gold" for Methods Development consisting of a Model 126 programmable module, a Model 168 diode array detector, a Model 210A injection valve with a 5 µL loop, a PC386SX (Wearnes) with "System Gold" version 5.1 software for data collection and controller function. A 120 x 4 mm Separon SGX RPS 5µm column (purchased from Tessek Ltd., Stranice, Czech Republic) and acetonitrile-0.1% trifluoroacetic acid (45:55) as the mobile phase with a flow rate 1 mL/min were used.

α- and γ-*tert*-Butyl *N*-benzyloxycarbonyl-L-glutamates.- To a solution of Z-Glu (56.25 g, 200 mmol) in dioxane (200 mL), cooled to 10°, conc. H_2SO_4 (2 mL) followed by liquid isobutene (200 mL) were added dropwise.The flask was well stoppered and the stopper was fastened so as to maintain excess pressure. The mixture was kept at 21-23° for 96 hrs then cooled to 10°, and excess isobutene was allowed to evaporate. Potasium carbonate (5.25g, 38 mmol) and then water (a total of 100 mL) in 10 mL portions was added (abundant foaming) and the dioxane evaporated. The residue was dissolved in a solution of K_2CO_3 (27.0 g, 200 mmol) in water (200 mL) and extracted with ether (3 x 100 mL; TLC R_f 0.90); the aqueous phase was acidified with 1N HCl to pH 3, and extracted with ether (3 x 100 mL); the combined extracts were washed with 0.01M K_2CO_3 (300 mL portions) until the Z-Glu (TLC R_f 0.10) was removed then with brine (3 x 100 mL) and evaporated. The residue was dried *in vacuo* for 24 hrs to give 40.4 g (60% yield) of monoesters. To a stirred solution of the monoesters in ether (860 mL), DCHA (24 mL, 120 mmol) was added dropwise over 30 min and the whole left standing overnight. The crystalline product was washed with cold ether (2 x 30 mL) and hexane (2 x 30 mL). The filtrate was concentrated at a temperature not exceeding 20° to 200 mL and after 1 hr standing, a second crop was collected and washed with cold ether (2 x 10 mL) and hexane (2 x 10 mL). A total of 34.43 g (33% yield) of Z-Glu-O*t*Bu•DCHA, mp. 146-147°, lit.[8,9] mp. 148-149°, was obtained. HPLC: tR = 4.09 min; 100% purity. The filtrate was concentrated to 100 mL, extracted successively with 0.5M citric acid (3 x 50 mL), cold water (3 x 50 mL) and brine (2 x 50 mL), evaporated, and the residue was dried *in vacuo* for 24 hrs and the oily solid was crystallized from ether-petroleum ether to give 14.96 g (22% yield) of Z-Glu(O*t*Bu), mp. 80-81°, lit.[10] mp. 85-86°, lit.[11] mp. 79-80°. R_f 0.45. HPLC: tR = 4.83 min; 99.8% purity. Z-Glu-O*t*Bu•DCHA, mp. 83-84°, lit.[6] 82-83.5°, can be converted to the free acid in 92% yield with $KHSO_4$ solution by using conventional procedure.[12] HPLC: tR = 4.09 min; 100% purity.

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1. Abbreviations used: Z = benzyloxycarbonyl, Glu = L-glutamic acid, *t*Bu = *tert*-butyl, Z-Glu(O*t*Bu) and Z-Glu-O*t*Bu = the γ and α- ester, respectively.

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