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SELECTIVE CLEAVAGE OF THE t-BUTYLOXYCARBONYL PROTECTING GROUP

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A disadvantage in the use of the t-butyloxycarbonyl(BOC) group[1,2] for N-protection in peptide synthesis is that the acidic conditions normally employed for its removal[3,4,5] also effect the cleavage of the t-butyl ester group. The latter is used mainly to protect the side chain carboxyl groups of aspartic and glutamic acids.[1,2] Selective protection of side chain carboxyl groups is of increasing importance for the synthesis of glycopeptides in which aspartic and glutamic acids are involved in peptide-sugar links.[6]

We wish to report a method by which the BOC-group can be removed under mild conditions which leave the t-butyl ester group intact. Hofmann et al.[7] reported that on the column of an amino-acid analyser, BOC-groups were completely hydrolysed but that γ-t-butyl glutamate was only partially hydrolysed. It therefore seemed probable that a similar ion-exchange resin might be used to bring about the selective cleavage of the BOC-group in the presence of the t-butyl ester group.

The removal of the BOC-group from BOC.Phe.Gly.OBu (I, m.p. 100-102°, $[\alpha]_D^{25}$ -8.0°(C= 1, MeOH), Rf_A 0.84; Found: C,63.7; H,7.6;

N,7.6. $C_{20}H_{30}N_2O_5$ requires C,63.4; H,7.9; N,7.4%)*, and from other protected peptides was carried out by the following general method. The compound, in a volume of MeOH/H_2O (1:1) equivalent to about 3/4 of the void volume of a column of Zeo-Carb 225 (H+), was applied to the column which, after 6 hr. at room temperature, was washed with MeOH/H_2O (1:1). This removed starting material. The product, retained on the column, was obtained by elution with MeOH/10% NH_4OH solution (1:1) followed by evaporation of the solvent. In this way, Phe.Gly.OBu (II, Rf_A 0.63, net positive charge: <u>picrate</u> - m.p. 83-85°, $[\alpha]_G^{25}$ +41.7° (C = 1.84, MeOH), Found: C,49.4;H,5.4; N,13.8. $C_{21}H_{25}N_5O_{10}$ requires C,49.7; H,4.9; N,13.8%) was obtained from I in 87% yield. I was completely deblocked by trifluoroacetic acid (TFA) to the free dipeptide (m.p. 71-73°, $[\alpha]_D^{25}$ +48.8° (C = 0.5, H_2O), Rf_A 0.5, net zero charge, Found: C,59.0; H,7.25; N,12.55. $C_{11}H_{14}N_2O_3$ requires C,59.2; H,6.7; N,12.25%).

Coupling of II with BOC.Ala.OH gave BOC.Ala.Phe.Gly.OBu (III, m.p. 110-111°, $[\alpha]_D^{25}$ -30.5°(C = 0.94, $CHCl_3$), Rf_A 0.89, Rf_B 0.94, Found:C,61.3; H,8.0; N,9.0. $C_{23}H_{34}N_3O_6$ requires C,61.6; H,7.6; N,9.4%) which was completely deblocked with TFA giving Ala.Phe.Gly.OH (m.p. 160-162°, $[\alpha]_D^{25}$ +45.2° (C = 0.6, MeOH), Rf_A 0.53, net zero charge, Found: C,52.5; H,6,9; N,12.9. $C_{14}H_{19}N_3O_4.1\frac{1}{2}H_2O$ requires C,52.6; H,6.8; N,13.1%. DNP.Ala.Phe.Gly.OH gave m.p. 66-68°, Found: C,52.4; H,4.8. $C_{20}H_{21}N_5O_8$ requires C,52.4; H,4.6%).

The BOC-group was selectively removed from III giving Ala.Phe.Gly.OBu (IV, Rf_A 0.76, net positive charge: <u>picrate</u>- m.p. 94-96°, $[\alpha]_G^{28}$ +90.0° (C = 0.5, MeOH), Found: C,50.4; H,5.0. $C_{24}H_{30}N_6O_{11}$ requires C,49.85; H,5.2%) in 47% yield. The protected tetrapeptide Z.Gly.Ala.Phe.Gly.OBu (Rf_A 0.88, Rf_B 0.91, $[\alpha]_D^{25}$ -7.0°

*Amino-acids, except glycine, were of the L-configuration. Thin-layer chromatography was carried out on MN-silica gel G: Rf_A, pyridine-HAc-BuOH-H_2O (10:5:35:50); Rf_B, BuOH-HAc-H_2O (4:1:5). Paper electrphoresis gave net charges at pH 7.0.

(C = 0.6, $CHCl_3$), amino-acid ratios in acid hydrolysate $Gly_{1.94}$ $Ala_{0.95}Phe_{1.10}$) was obtained in 65% yield when IV was coupled with Z.Gly.OH by the mixed anhydride method.[2]

Selective deprotection of BOC.Ala.Glu.$(OBu)_2$ (V, m.p. 87-89°, $[\alpha]_D^{25}$ - 27.0° (C = 1.2, MeOH), Rf_A 0.90, Found: C, 58.8; H, 8.8; N, 6.3. $C_{21}H_{38}N_2O_7$ requires C, 58.6; H, 8.8; N, 6.5%) gave a 63% yield of Ala.Glu.$(OBu)_2$ (VI, Rf_A 0.74, net positive charge: picrate - m.p. 70-72°, Found: C, 46.8; H, 5.9. $C_{22}H_{33}N_5O_{12}$ requires C, 47.4; H, 5.9%) while TFA converted V into the free dipeptide Ala.Glu.$(OH)_2$ (m.p. 92-98°, $[\alpha]_D^{24}$ - 40.0° (C = 0.5, MeOH), Rf_A 0.11, net negative charge, Found: C, 40.9; H, 5.3; N, 11.6. $C_8H_{14}N_2O_5.H_2O$ requires C, 40.6; H, 5.9; N, 11.8%. DNP-Ala.Glu.$(OH)_2$ had m.p. 75-77°, Rf_A 0.36, Found: C, 43.8; H, 4.2. $C_{14}H_{16}N_4O_9$ requires C, 43.7; H, 4.7%).

From VI was prepared BOC.Phe.Ala.Glu.$(OBu)_2$ (VII, m.p. 76-78°, $[\alpha]_D^{25}$ - 31.3° (C = 1.5, MeOH), Rf_A 0.93, Rf_B 0.95, Found: C, 61.7; H, 8.1; N, 7.6. $C_{30}H_{47}N_3O_8$ requires C, 62.3; H, 8.1; N, 7.2%), which was completely deblocked with TFA giving the free tripeptide Phe.Ala.Glu.$(OH)_2$ (m.p. 153-156°, $[\alpha]_D^{25}$ - 27.0° (C = 1.0, MeOH), Rf_A 0.54, net negative charge, Found: C, 53.9; H, 5.9. $C_{17}H_{23}N_3O_6$ $\frac{1}{2}H_2O$ requires C, 54.5; H, 6.4%). Selective removal of the BOC group from VII gave the partially protected tripeptide Phe.Ala.Glu.$(OBu)_2$ (VIII, $[\alpha]_D^{25}$ - 15.0° (C = 0.6, benzene), Rf_B 0.54, net positive charge: picrate - m.p. 110-111°, Found: C, 51.5; H, 6.1; N, 11.6. $C_{31}H_{42}N_6O_{13}$ requires C, 51.5; H, 5.8; N, 11.7%) in 72% yield, and VIII was coupled with Z-(nitro)Arg.Ala.OH[8] by the mixed anhydride method. This gave the protected pentapeptide Z-(nitro) Arg.Ala.Phe.Ala.Glu.$(OBu)_2$,$[\alpha]_D^{25}$ - 15.0° (C = 0.5, MeOH), Rf_B 0.95, paper chromatography - Rf_A 0.93, Rf_B 0.96. On acid hydrolysis the pentapeptide gave amino-acid ratios: $Nitroarg_{1.06}Ala_{2.03}Phe_{0.95}$ $Glu_{0.97}$ (nitroarginine analysed as ornithine and arginine).

The method therefore promises to extend the usefulness of

the BOC and t-butyl ester groups for glycopeptide synthesis and for organic chemistry in general.

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REFERENCES

1 E. Schroder and K. Lubke, "The Peptides", Vol. 1, Academic Press, New York, 1965.

2 K. Hofmann and P.G. Katsoyannis in "The Proteins", ed. H. Neurath, 2nd. Ed., Vol.1, p.53, Academic Press, New York, 1963.

3 G.W.Anderson and A.C.McGregor, J.Am.Chem.Soc. 79, 6180, (1957).

4 F.C.McKay and N.F.Albertson, J.Am.Chem.Soc.,79, 4686, (1957).

5 R.Schwyzer and H. Kappeler, Helv.Chim.Acta. 44, 1991 (1961); H.Kappeler and R.Schwyzer, ibid, 43, 1453 (1960).

6 "Glycoproteins", ed. A. Gottschalk, Vol. 5, Elsevier, Amsterdam, 1966, p. 273.

7 K.Hofmann, R. Schmiechen, R.D.Wells, Y.Wolman and N.Yanihara, J.Am.Chem.Soc., 87, 611 (1965).

8 K.Hofmann, W.D.Peckham and A.Rheiner, J.Am.Chem.Soc., 78, 238 (1956).