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SELECTIVE CLEAVAGE OF N-t-BUTOXYCARBONYL PROTECTING GROUP

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The selective cleavage of BOC-peptide-OBu^t was investigated by the use of 85% formic acid, and it was found that BOC-peptide-OBu^t gave H-peptide-OBu^t in good yield by this procedure. This method was also applied to investigation of the selectivity of di-t-butyl esters of dibasic amino acids and N-deprotection of peptides containing these amino acids.

The deblocking of the t-butoxycarbonyl(BOC) groups and the cleavage of the O-t-butyl ester(OBu^t) functions of peptides with formic acid were reported by Halpern and Niteki.[1)]

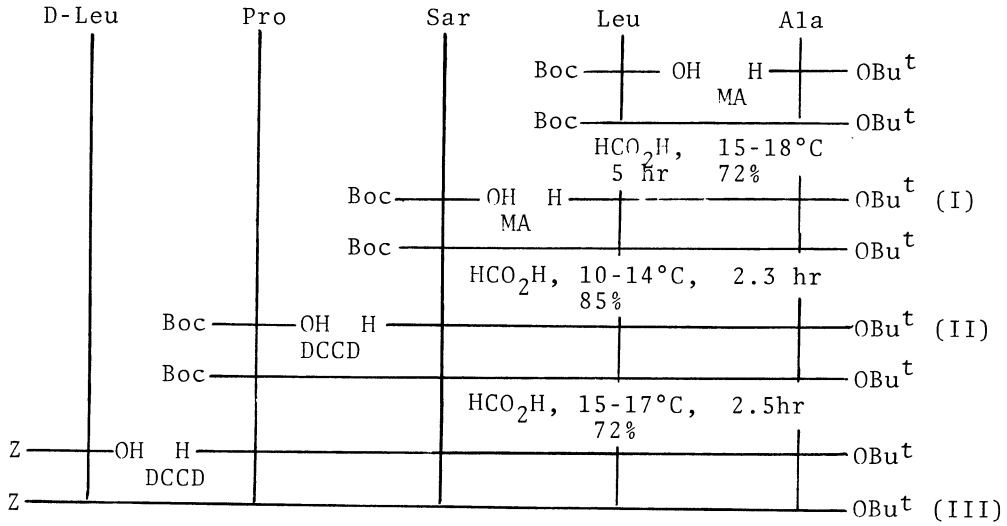
In our preliminary experiments we have found that the t-butoxycarbonyl group of amino acid is cleaved faster than the t-butyl ester group of amino acid by the use of 85% formic acid(commercially available). It therefore seemed that this difference in reactivity might be used to bring about the selective cleavage of the t-butoxycarbonyl group in the presence of the t-butyl ester group.

We wish to report a method by which the t-butoxycarbonyl group can be removed under appropriate conditions which leave the t-butyl ester intact. Although the selective cleavage of the t-butoxycarbonyl groups was reported by Gray and Khoujah using ion exchange resin,[2)] our method seemed to be simpler and practically more useful.

The removal of the BOC group from BOC-peptide-OBu^t was carried out as follows. The reaction was followed by t.l.c. In a typical experiment BOC-Phe-Gly-OBu^t (400 mg)(mp 107-108°C, $[\alpha]_D$ -8.1°(MeOH), Found: C,63.60; H,7.85; N,7.26. Calcd for $C_{20}H_{30}O_5N_2$: C,63.47; H,7.99; N,7.40%) was dissolved in cold 85% formic acid (17 ml), and the solution kept at 15-17°C for 150 minutes. Water was added to

this mixture, and the aqueous solution was evaporated in vacuo(bath temp. 30°C). The residual oil was dissolved in minimum amount of water, and the unreacted material was extracted with ethyl acetate. Sodium hydrogen carbonate was added to the residual aqueous solution to bring about alkaline,and the isolated oil was extracted with ethyl acetate. By this procedure analytically pure H-Phe-Gly-OBu^t (oxalate: mp 167-168°C, $[\alpha]_D$ +26.5°(abs. EtOH), Found: C,55.07; H,6.44; N,7.45. Calcd for $C_{17}H_{24}O_7N_2$: C,55.43; H,6.56; N,7.61%.) was obtained in an 80% yield. In a similar manner BOC-Ala-Phe-Gly-OBu^t (mp 149-151°C, $[\alpha]_D$ -42.5°(abs. EtOH), Found: C,61.18; H,7.69; N,9.07. Calcd for $C_{23}H_{35}O_6N_3$: C,61.45; H,7.85; N,9.35%.) was converted to H-Ala-Phe-Gly-OBu^t(oxalate: mp 164°C, $[\alpha]_D$ -6.7°(abs. EtOH), Found: C,54.46; H,6.60; N,9.41. Calcd for $C_{20}H_{29}O_8N_3$: C,54.66; H,6.65; N,9.56%.) in a 77% yield. Benzyloxycarbonyl(Z)-pentapeptide t-butyl ester was synthesized using this method(Scheme 1)

Scheme 1



MA: Mixed Anhydride Method

DCCD: Dicyclohexylcarbodiimide

The analytical values and physical constants of I,II and III were shown below.

I(oxalate): mp 176-177°C, $[\alpha]_D$ -18.5°(abs. EtOH), Found: C,51.66; H,7.89; N,7.96. Calcd for $C_{15}H_{28}O_7N_2$: C,51.71; H,8.10; N,8.04%.

II(oxalate): mp 195°C, $[\alpha]_D$ -32.5°(DMF), Found: C,51.40; H,8.10; N,9.89. Calcd for $C_{18}H_{33}O_8N_3$: C,51.54; H,7.93; N,10.02%.

III: mp 80°C, $[\alpha]_D$ -21.7°(abs. EtOH), Found: C,62.76; H,8.10; N,10.76. Calcd

for $C_{35}H_{55}O_8N_5$: C,62.41; H,8.17; N,10.40%.

The physical constants of I and II(oxalates) were identical, respectively, with those of H-Leu-Ala-OBu^t oxalate[mp 176°C, $[\alpha]_D$ -17.7°(abs. EtOH)] and H-Sar-Leu-Ala-OBu^t oxalate[mp 195°C, $[\alpha]_D$ -32.2°(DMF)] prepared by the debenzyloxycarbonylation of corresponding Z-peptide-OBu^t. These results indicate that there is no racemization during the processes.

Selective deprotection of Z-Glu-(OBu^t)$_2$ and Z-Asp-(OBu^t)$_2$

Z-Glu-(OBu^t)$_2$ was dissolved in cold 85% formic acid and kept at 18°C for 130 minutes. The reaction mixture was diluted with water, and the isolated oil was extracted with ethyl acetate. After the organic layer was neutralized with $NaHCO_3$ aqueous solution, the solvent was removed in vacuo. The residual oil was chromatographed on silica gel. By this procedure we have obtained Z-Glu-OBu^t (DCHA salt, mp149-150°C)[3] in a 50% yield. Under these reaction conditions only γ-OBu^t was selectively removed, and from this result this method seemed to be useful for the synthesis of γ-peptide of glutamic acid.

In the same manner di-t-butyl benzyloxycarbonylaspartate gave Z-Asp-OBu^t(DCHA salt: mp 108-109.5°C, $[\alpha]_D$ -0.96°(abs. EtOH), Found: C,66.60; H,8.81; N,5.28. Calcd for $C_{28}H_{44}O_6N_2$: C,66.64; H,8.79; N,5.55%) in a 40% yield after the isolation by column chromatography on silica gel(reaction time 3 hr, reaction temp. 18°C).

Selective deprotection of BOC-Leu-Asp-(OBu^t)$_2$ and BOC-Ala-Glu-(OBu^t)$_2$.

Di-t-butyl t-butoxycarbonyl-leucyl-aspartate(BOC-Leu-Asp-(OBu^t)$_2$)(270 mg) was dissolved in 15 ml of cold 85% formic acid and kept at 12°C for 3.5 hr. To the reaction mixture was added 5 ml of water, and the resultant mixture was concentrated to dryness in vacuo at 26°C. The residual oil was dissolved in ethyl acetate. The organic layer was washed with a small excess of 10% aqueous $NaHCO_3$ solution to neutralize the formate and washed with water. H-Leu-Asp-(OBu^t)$_2$ (oxalate: mp 165°C, $[\alpha]_D$ -7.02°(abs. EtOH), Found: C,53.29; H,7.97; N,6.33. Calcd for $C_{20}H_{36}O_9N_2$: C,53.56; H,8.09; N,6.25%) was obtained in a 66% yield after the isolation by column chromatography on silica gel.

BOC-Ala-Glu-(OBu^t)$_2$ was converted to H-Ala-Glu-(OBu^t)$_2$(oxalate: mp 134-136°C, $[\alpha]_D$ -13.0°(abs. EtOH), Found: C,50.49; H,7.61; N,6.39. Calcd for $C_{18}H_{32}O_9N_2 \cdot \frac{1}{2}H_2O$: C,50.34; H,7.75; N,6.52%) in a 50% yield by a similar method(reaction time 4 hr, reaction temp. 8-10°C).

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References

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