# Solid-phase Synthesis of Tryptophan-containing Peptides

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*Summary* A new method for the cleavage of the t-butoxycarbonyl group in the solid-phase synthesis of tryptophan-containing peptides has been developed, and its effectiveness demonstrated in the synthesis of Lys-Ala-Gly-Leu-Gly-Trp-Leu.

IN solid-phase peptide syntheses, it is well known that tryptophan in the peptide chain growing on the polymer support undergoes oxidation during treatment with HCl in AcOH or dioxan for cleavage of the $Bu^tO{\cdot}CO$ group. In such cases, $HO{\cdot}CH_2{\cdot}CH_2{\cdot}SH$[1] or $HS{\cdot}CH_2{\cdot}[CHOH]_2{\cdot}CH_2{\cdot}SH$[2] has been used as a scavenger to protect tryptophan from oxidation. Bubbling of HCl into a solution of tryptophan in formic acid results in formylation of the indole nitrogen ($N^1$) and this formyl group can be removed in a weakly alkaline medium.[3] Further observations on the essential stability of tryptophan and $N^1$-formyltryptophan in 0·1—1·0N-HCl-$HCO_2H$, together with rapid cleavage of the $Bu^tO{\cdot}CO$ group with a 2—10 fold molar excess of HCl in $HCO_2H$ and removability of $N^1$-formyl group in dimethylformamide (DMF) containing a 20—30 fold molar excess of hydrazine hydrate led us to examine HCl–$HCO_2H$ as a reagent for cleaving the $Bu^tO{\cdot}CO$ group in the solid-phase synthesis of tryptophan-containing peptides.

As an example, a 6-fold molar excess of HCl (0·1N) in $HCO_2H$ was used in the synthesis of Lys-Ala-Gly-Leu-Gly-Trp-Leu,† which was constructed as usual[1,2] starting from $Bu^tO{\cdot}CO$–leucyl resin. In parallel, two peptides with the same sequence were prepared using 1N-HCl–AcOH in the presence and absence of $HO{\cdot}CH_2{\cdot}CH_2{\cdot}SH$ (2%). Half of each protected peptide resin was cleaved by hydrazine hydrate–DMF as described.[4] The products (**1a**—**c**) [from $Bu^tO{\cdot}CO$-Lys(ε-Z)-Ala-Gly-Leu-Gly-Trp‡-Leu-$NHNH_2$] (Z = $PhCH_2{\cdot}O{\cdot}CO$) were reprecipitated from the methanolic solutions by addition of $Et_2O$–light petroleum (1 : 2v/v); (**1a**) (*via* HCl–$HCO_2H$) had m.p. 198—200°, $[\alpha]_D^{20}$ −28° (MeOH) [86% of the yield of (**1b**)]; (**1b**) (*via* HCl–AcOH–$HO{\cdot}CH_2{\cdot}CH_2{\cdot}SH$) had m.p. 189—191°, $[\alpha]_D^{20}$ −22°; and (**1c**) (*via* HCl–AcOH) had m.p. 181—184°, $[\alpha]_D^{20}$ −13°. The other halves of the peptide resins were treated with anhydrous HF in the presence of anisole; the peptide produced *via* HCl–$HCO_2H$ was further treated with 0·1N-aqueous piperidine to remove the $N^1$-formyl group attached to a part of the tryptophyl residue since this group is resistant to anhydrous HF as well as anhydrous $Et_3N$ in DMF in the neutralization step. (**2a**) from HCl–$HCO_2H$ was an amorphous white powder and (**2b**) from HCl–AcOH–$HO{\cdot}CH_2{\cdot}CH_2{\cdot}SH$ was an amorphous light brown powder.

† All amino-acids have the L-configuration except for glycine.

‡ May or may not involve pure tryptophyl residue.

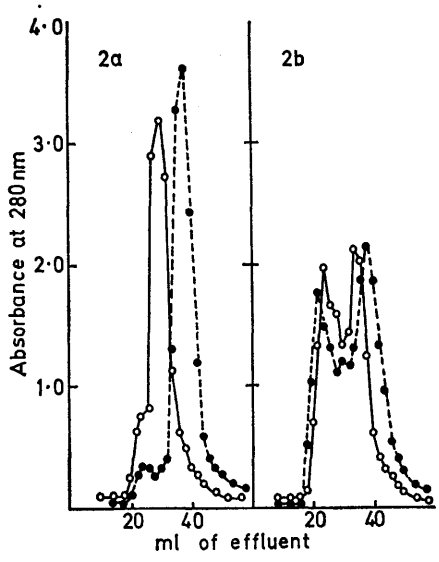


FIGURE. *Elution diagrams of the peptides* (**2a**) (6 mg) *and* (**2b**) (6 mg), *on a Sephadex G-25 column* (0·9 × 60 *cm*) *before* (○—○) *and after* (●- - -●) *modification with* NCPS-Cl; *solvent*, 10% AcOH.

The u.v. spectrum of (**1a**) was virtually identical with that of Z-tryptophan except that a trough in the 240—250 nm region showed somewhat greater absorption and was shifted to the red by 2 nm, indicating that (**1a**) was contaminated to only a minor extent by oxidized species. The extinction coefficients of (**1b**) and (**1c**) at 282 nm were respectively 34 and 45% more intense than that of (**1a**), possibly owing to oxidation of the indole nucleus, although the products have not yet been identified.

The Figure shows the gel-chromatographic patterns of (**2a**) and (**2b**) on a Sephadex G-25 column. (**2b**) gave a complex profile owing to its heterogeneity. To ascertain whether the major peak from (**2a**) is due only to pure peptide, (**2a**) was treated with a 10-fold molar excess of 2-nitro-4-carboxyphenylsulphenyl chloride (NCPS-Cl)[5] in 80% $HCO_2H$ and the modified peptide was chromatographed on the same column. It showed an increased adsorptivity on the gel compared with the unmodified peptide,[6] indicating that the major peak for (**2a**) involved only pure tryptophyl residue. Comparison of the two elution diagrams before and after reaction of (**2b**) with NCPS-Cl indicates that (**2b**) contains unchanged tryptophan to a smaller extent.

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