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A NON-DESTRUCTIVE METHOD FOR THE DETERMINATION OF COMPLETENESS OF COUPLING REACTIONS IN SOLID PHASE PEPTIDE SYNTHESIS

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Frequently, one desires to know if a coupling reaction in the Merrifield solid phase peptide synthesis (1) has proceeded to completion before continuing the synthesis. Incomplete coupling reactions may lead eventually to the formation of peptide fragments with missing amino acids in addition to the desired peptide (2).

Two principal ways exist presently for quantitative determination of completeness of solid phase peptide coupling reactions. These involve, (a) sacrifice of a small sample of the resin peptide for hydrolysis and subsequent amino acid analysis (3), and, (b) spectroscopic measurement of a soluble product formed in the reaction, e.g. measurement of p-nitrophenol formed in p-nitrophenyl ester coupling (4)(5). Both methods have some serious drawbacks. The former requires a considerable amount of time; the latter is obviously limited to active ester coupling and even further limited to active esters containing chromophoric leaving groups.

We wish to describe a simple, non-destructive procedure for quantitatively determining the completeness of solid-phase coupling reactions in conjunction with the use of N-t-butyloxycarbonyl (BOC) protected amino acids and active esters. Following the coupling reaction (ca. 1 mmole scale) and its subsequent washing steps, the resin peptide is (a) washed* (3 x 1 min.) with methylene chloride, (b) treated with 20-25 ml of 0.3 M pyridine hydrochloride (Pyr·HCl) in dry methylene chloride** to form the hydrochloride of any uncoupled resin amine, (c) washed (3 x 2 min.) with methylene chloride, (d) washed (3 x 2 min.) with dimethylformamide, (e) treated with 2 ml of triethylamine in 20 ml of dimethylformamide for 3 min. to neutralize any hydrochloride formed in (b), and (f) washed with diemthylformamide. The filtrates of (e) and (f) are

*Washing portions 20-25 ml.

**Ca. 4 g of pyridine hydrochloride, tech. grade, is dissolved in 100 ml of methylene chloride and the solution is dried over Drierite® and filtered. The concentration of Pyr·HCl is determined by evaporating an aliquot and titrating the residue with standard silver nitrate.

combined, chilled, acidified with excess dilute nitric acid and titrated potentiometrically with standard silver nitrate. The equivalents of silver nitrate required represent the amount of uncoupled resin amine (the amount of resin amine present before coupling can be determined in the usual manner (6)).

The success of this method rests on the ability of pyridine hydrochloride to form quantitatively the hydrochloride of uncoupled resin amine without causing premature removal of the t-butyloxycarbonyl protective group of the coupled amino acid. These criteria were met by this reagent as determined in the following ways. BOC-Gly-O-Resin (1.07 mmols Gly/g resin) was essentially unaffected by a 15-min. treatment with 0.3 M Pyr·HCl in methylene chloride. No change in the n.m.r. spectrum of BOC-alanine, 15% in 0.30 M Pyr·HCl in methylene chloride, was detectable after 20 hr. at room temperature. Resin amine, H-Gly-O-Resin, was quantitatively converted to its hydrochloride form by a 3-min. treatment with 0.3 M Pyr·HCl/CH_2Cl_2. The ΔpK between pyridine and a typical amino acid amide or ester is ca. 2.78 and 2.60, respectively, based on the pK values of glycinamide (6.07)(7) and methyl glycinates (6.25)(7) and pyridine (8.85).

Finally, the use of 0.3 M Pyr·HCl/CH_2Cl_2 in a solid phase peptide synthesis was demonstrated. The tetrapeptide, H-Leu-Ala-Gly-Val-OH (7), was synthesized starting with high capacity BOC-Val-O-Resin (2.02 mmoles Val/g resin)(9). Typical procedures were used in the deblocking, washing, neutralization and coupling (DCCI) steps (1). Pyridine·HCl/CH_2Cl_2 reagent revealed that the BOC-glycine coupling step was essentially complete ($\geq$99.5%) and the BOC-alanine step 93% complete. Before proceeding to the next cycle the BOC-alanine coupling reaction was repeated raising the level of coupling to $\geq$99.1%. The terminal BOC-leucine coupling step proceeded only to the extent of 69%. This step was not repeated; instead the peptide mixture was removed from its resin support with HBr/trifluoroacetic acid. An analysis of the mixture using an automatic amino acid analyzer which was also adapted for analysis of peptides of this model system showed no measurable amount of valine or dipeptide, H-Gly-Val-OH. Only the tripeptide H-Ala-Gly-Val-OH (38%) and tetrapeptide (62%) were shown to be present. The difference between the relative amounts of tri- and tetrapeptide attached to and removed from the resin is probably a reflection of the heterogeneity of resin sites (5).

Unfortunately, Pyr·HCl/CH_2Cl_2 cleaves the o-nitrophenylsulfenyl (NPS) protective group at a substantial rate, thereby precluding the use of this reagent with the NPS protective group.

Pyridine hydrochloride solution had no observable affect on tryptophan residues when used in conjunction with mercaptoethanol (10).

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