Reactivity of *p*-Nitrophenyl Esters in Solid Phase Peptide Synthesis

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Up to now several reports on the use of *p*-nitrophenyl esters (—ONp) of *t*-butyloxycarbonyl- (Boc-) amino acids for solid phase peptide synthesis (SPPS) have appeared, most of which were discussed by Merrifield [1] recently. Although obviously not ideal in practice for SPPS, activated esters, including the hitherto most popular *p*-nitrophenyl esters, are important in this context mainly because dicyclohexylcarbodiimide cannot be used for incorporation of asparagine and glutamine, owing to the formation of by-products. The economic aspect with regard to the fact that excess activated ester can be recovered but excess amino acid in the carbodiimide procedure cannot, is certainly less important.

The present confusion in the field of SPPS concerning protecting groups, the methods for their removal and especially the efficiency of certain coupling methods, we believe is caused by the fact that mainly *qualitative* methods, if any, have been used to verify that individual steps have proceeded satisfactorily. This means in practice that every step must have a nearly *quantitative* yield, the tolerable deviation depending, of course, on the size of the desired peptide.

Merrifield [2] found that *p*-nitrophenyl-esters were inferior to dicyclohexylcarbodiimide for coupling in SPPS. Bodanszky and Sheehan [3,4] showed that *p*-nitrophenyl esters really reacted and prepared a protected hexapeptide methyl ester. Recently Bodanszky and Bath [5] determined half reaction times for three different activated esters, including a *p*-nitrophenyl esters, with glycine and valine, fixed to resins.

This communication presents actual figures on the efficiency of coupling a *p*-nitrophenyl ester to amino groups present on resins. Our intention was to find out how rapidly and especially how completely the reaction proceeded, since this is the crucial point. We used the method [6] recently developed in this department for measuring quantitatively the amount of primary amino groups on a Merrifield resin. All experiments were performed with Boc-L-asparagine *p*-nitrophenyl ester and all but a few with L-leucyl-L-alanyl-polymer. The latter was obtained from Boc-L-leucyl-L-alanyl-polymer.[7] Additional resins were prepared similarly. As can be seen below, all experiments except the last one were done on a small scale under considerable dilution. This last fact is to some extent reflected in the results compared to the final experiment.

From the experiments performed we conclude that *p*-nitrophenyl esters, even when present in very great excess, react slowly. This is especially the case when compared with dicyclohexylcarbodiimide for coupling.[6] Consequently, in order to ensure a satisfactory reaction very long contact times are necessary. Carbodiimide certainly gives faster and more complete coupling when applicable.

Procedure and results. A: Small scale experiments. About 100 mg of L-Leu-L-Ala-polymer, obtained from Boc-L-Leu-L-Ala-polymer [7] by deprotection with trifluoroacetic acid-methylene chloride 1:1 (v/v), rinsed, neutralized and dried, were reacted with 5 equiv. of Boc-L-Asn-ONp in 4 ml of DMF in sintered glass filter vessels for different times. The liquid was then sucked off and the polymer washed twice with DMF, once with glacial acetic acid, three times with abs. ethanol and once with methylene chloride, with 2—5 min rotation each time. The resin, suspended in abs. ethanol-methylene chloride 1:1 (v/v), was allowed to react for 12 h with a great excess (50—100 times) of 2-hydroxy-1-naphthaldehyde at room temperature. After removal of the aldehyde solution and careful washing of the polymer, excess benzylamine in methylene chloride was added and allowed to react for 30 min. The solution was collected together with further methylene chloride and ethanol used for rinsing the resin and, after dilution with ethanol, the amount of Schiff base was determined spectrophotometrically at 420 nm. The molar absorptivity of the Schiff base at 420 nm has been determined [8] to be 10 040 in ethanol.

The following values for the amount of free amino groups after coupling, expressed in per cent of the quantity originally present, were obtained:

After	1 h	30 %
	5 h	10 %
	17.5 h	0.4 %

Further experiments with the amount of Boc-L-Asn-ONp (I) below and the same polymer under similar conditions gave:

With 2 × 5 equiv. of I for 2 × 2.5 h 4 %
10 equiv. of I for 5 h 5 %
5 equiv. of I for 1 h in DMF-methylene chloride 1:3.6 63 %

With Gly-L-Ala-polymer and 5 equiv. of I for 5 h 6 %

With L-Ile-L-Ala-polymer * and 5 equiv. of I for 5 h 5 %

B: Preparative experiment. 2.4 g of L-Leu-L-Ala-polymer, corresponding to about 0.6 mmole of dipeptide, were swollen in DMF. 1.06 g (3.0 mmole) of Boc-L-Asn-ONp in 5 ml of DMF were added and allowed to react for 5 h under rotation. The DMF solution was sucked off and the resin rinsed twice with DMF and twice with abs. ethanol (as before). A small sample was withdrawn and dried, and the amount of free amino groups still present as determined with the above procedure was found to be 3 %. Submitting the resin to a second condensation with 1.06 g of Boc-L-Asn-ONp in the same amount of DMF for a further 5 h and proceeding as just mentioned, reduced the amount of unreacted amino groups to 0.9 %.

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* L-Ile-L-Ala-polymer was reacted without prior drying.

Reactions between *p*-Benzoquinone and Pyrogallol

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The acid-catalysed reaction between *p*-benzoquinone and suitable benzene derivatives sometimes offers a convenient synthetic route to *p*-terphenyls.[1-4] Aluminium chloride has often been employed as a catalyst but other acids may also be used to effect this type of reaction. Pummerer and Huppmann obtained a termolecular product on reacting *p*-benzoquinone and resorcinol in dilute sulphuric acid.[5-6] However, this reaction has been shown to give an *m*-terphenyl derivative.[7]

This finding of the formation of an *m*-terphenyl prompted us to investigate similar reactions between *p*-benzoquinone and phenols in dilute sulphuric acid. In this paper, we wish to report some results obtained during an investigation of the reactions between *p*-benzoquinone and pyrogallol.

A mixture of *p*-benzoquinone and pyrogallol was treated with dilute sulphuric acid for two days. The dark reaction mixture was extracted with ethyl acetate and the extract treated with aqueous sodium dithionite. The phenolic mixture obtained by this procedure was methylated with dimethyl sulphate. The mixture of methyl ethers was steam-distilled in order to remove large amounts of pyrogallol trimethyl ether and hydroquinone dimethyl ether. The residue was distilled *in vacuo* and two fractions were obtained.

According to thin layer chromatography, the low-boiling fraction consisted of three compounds. These were separated by chromatography on a preparative scale. It was assumed that one of these compounds was formed by simple combination of *p*-benzoquinone and pyrogallol and this was found to be the case. 2,3,4,2',5'-Pentamethoxybiphenyl was synthesized by reacting *p*-benzoquinone with the diazonium salt from 2,3,4-trimethoxyaniline according to the