Use of Esters of 2,5-Diphenyl-2,3-dihydro-3-oxo-4-hydroxythiophene Dioxide in Solid Phase Peptide Synthesis. A New Procedure for Attachment of the First Amino Acid

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The title esters derived from fluorenylmethoxycarbonyl amino acids undergo transesterification with hydroxymethyl polymers in the presence of tertiary base without racemisation.

As part of a continuing survey of activated *N*-fluorenylmethoxycarbonyl (Fmoc) amino acid derivatives suitable for use in solid phase peptide synthesis,[1,2] we have recently examined esters (**2**) of 2,5-diphenyl-2,3-dihydro-3-oxo-4-hydroxythiophene dioxide (TDO).[3] These TDO esters are readily prepared by reaction of the cyclic carbonate (**1**) with the carboxylic acid.[3,4] In the presence of one equivalent of base, esters (**2**) enolise with formation of the deep red anion (**3**) in which the enolate oxygen is well placed to facilitate aminolysis (**3**, arrows)‡ or alcoholysis. As expected for this mechanism, we find that aminolysis takes place more rapidly in non-polar media (dichloromethane) than in the polar dimethylformamide. We draw attention now to their particular efficiency in esterification of the first amino acid residue to the resin support *without any evidence for significant racemisation of chiral centres*. The concomitant colour changes also provide the first opportunity for automatically monitoring[6] this key step in solid phase synthesis.

Efficient esterification of activated Boc- and Fmoc-amino acids to hydroxymethyl resins in both the polyamide and polystyrene series has commonly required use of the powerful acylation catalyst 4-dimethylaminopyridine. Some years ago we drew attention to the potentially serious racemising properties of this basic reagent towards activated *N*-protected amino acids, even when the protecting group was a racemisation-resistant urethane derivative.[7] Dimethylaminopyridine is not required in the present technique.

In experiments using polydimethylacrylamide resin[8] functionalised by prior attachment of the acid-labile linkage agent (**4**), Fmoc-Ala-OTDO and the similar leucine derivative were transesterified to the resin at 40 mM concentration in dichloromethane containing 1 equiv. of di-isopropylethylamine in 1 h in yields of 94 and 93% respectively.§ In contrast, the corresponding pentafluorophenyl esters reacted in the presence of dimethylaminopyridine in the same time to less than 30%. Other representative TDO esters were also

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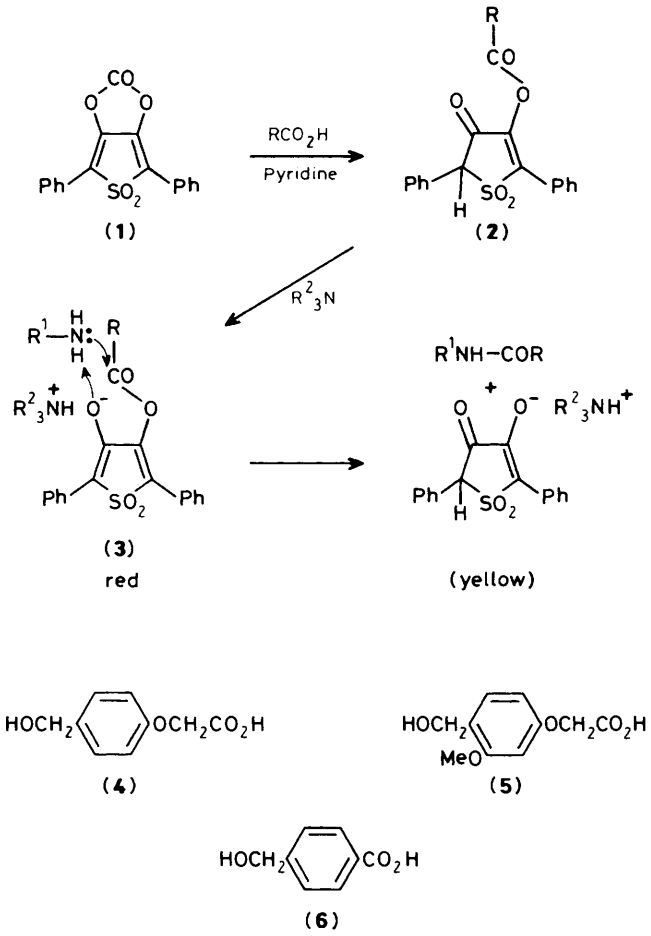
‡ *Cf.* the similar aminolysis of monoesters of *o*-catechol.[5]

§ Yields were determined by further reaction of remaining resin-bound hydroxyl groups with a large excess of Fmoc-Gly-OTDO, cleavage from the resin, and h.p.l.c. analysis of the liberated Fmoc-amino acid/Fmoc-glycine mixture. Some resin samples were also checked by amino acid analysis.



incorporated efficiently, *e.g.* Ser(Bu^t) 89%, 2 h; Asp(OBu^t) 95%, 1 h; Arg(Mtr) 81%, 1 h; Ile 89%, 10 h; Tyr(Bu^t) 97%, 1 h; Cys(Bu^t) 97%, 2 h. Resins functionalised with linkage agents (**5**) and (**6**) were similarly esterified efficiently (96 and 94% Fmoc-alanine incorporated after 1 h), and the polystyrene resin of Wang[9] reacted quantitatively in the same time.

Three separate racemisation tests were carried out. No formation of D-alloisoleucine was detected by amino acid analysis when Fmoc-L-isoleucine TDO ester was coupled to polydimethylacrylamide functionalised with (**4**).[7] No significant diastereoisomer was detected by h.p.l.c. when Leu and Ile resins¶ were treated with Marfey's reagent,[10] or when Ala, Ile, and Ser(Bu^t) resins were treated with Boc-L-Leu-ONp.[11]

We conclude that the Fmoc-amino acid TDO esters offer real promise for efficient racemisation-free esterification of protected amino acids to hydroxymethyl resins. The reaction is accompanied by a decrease in absorption in the visible spectrum at 500 nm which may be used for monitoring both ester and peptide bond forming reactions. When combined with our previous studies on monitoring of acylation and deprotection steps,[6,12] and feedback control of synthesis,[6] it offers prospect of true automation in all stages of solid peptide synthesis.

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¶ Nor with Ala, Tyr(Bu^t), or Ser(Bu^t), though D- or DL-amino acid derivatives were not available to verify expected elution positions.