Racemisation of Activated, Urethane-protected Amino-acids by *p*-Dimethylaminopyridine. Significance in Solid-phase Peptide Synthesis

BY ERIC ATHERTON, N. LEO BENOITON,† EVELYN BROWN, ROBERT C. SHEPPARD, and BRIAN J. WILLIAMS
(*M.R.C. Laboratory of Molecular Biology, Hills Road, Cambridge* CB2 2QH)

Summary Racemisation of N_α-t-butoxycarbonyl, fluoren-9-ylmethoxycarbonyl, and benzyloxycarbonyl amino-acid anhydrides by *p*-dimethylaminopyridine is shown to be a significant side reaction during attachment of the first amino-acid to the resin in solid-phase peptide synthesis.

p-DIMETHYLAMINOPYRIDINE is an efficient catalyst for acylation of hydroxy-groups.[1] A currently important application is in the esterification of the first (carboxy terminal) *N*-protected amino-acid residue to hydroxymethyl polymers in solid-phase peptide synthesis (*e.g.* refs. 2—5). In our recent syntheses[3a] of 13, 14, and 17 residue gastrin peptides by the polyamide solid-phase method[6] we observed formation in each case‡ of a minor (*ca.* 4%) impurity separable by preparative h.p.l.c. These impurities were apparently isomeric in amino-acid composition with the parent gastrins but were incompletely degraded by enzymes in the carboxy terminal (—Asp.Phe.NH_2) region. The stability of β-t-butyl aspartate residues to $\alpha \rightarrow \beta$ rearrangement under the conditions of peptide synthesis has been convincingly demonstrated.[6c,7] In view of recent developments[8,9] showing that, contrary to long held beliefs, oxazolone formation and hence racemisation of urethane-protected α-amino-acids is indeed possible, we considered that these impurities might contain carboxy terminal D-phenylalanine. The experiments described below show that appreciable racemisation does occur under reaction conditions commonly used for esterification of the first amino-acid to hydroxymethyl polymers.

An excess of the symmetrical anhydride of *N*-t-butoxycarbonyl-L-isoleucine prepared in dichloromethane in the usual manner[6b] and dissolved in dimethylacetamide was added to *p*-hydroxymethylphenoxyacetyl-polydimethylacrylamide resin[6c,10] in dimethylacetamide containing an equivalent amount of *p*-dimethylaminopyridine. After 5 h the resin was washed and bound amino-acid liberated and deprotected by a single treatment with 95% aqueous trifluoroacetic acid. Amino-acid analysis[11] showed that the isoleucine contained 5% of the *allo*-isomer. The observed racemisation was nearly independent of solvent (dimethylformamide, dichloromethane) or urethane *N*-protecting group (fluorenylmethoxycarbonyl or benzyloxycarbonyl) (see Table). When the anhydride and dimethylaminopyridine were mixed and kept for 5 min before addition to the polymer, 20% of *allo*-isoleucine was obtained. Racemisation was reduced substantially by adding the dimethylaminopyridine to the resin *after* the anhydride, by shortening the coupling time, and particularly by reducing the amount of pyridine derivative added. Under the original conditions, fluorenylmethoxycarbonyl-L-leucine formed 1·5% of the D-isomer, and t-butoxycarbonyl-L-phenylalanine gave 2%. *p*-Dimethylaminopyridine had no effect on the isomer content or absolute amount of Boc-isoleucine previously bound to the resin support.§

Best conditions thus far found for efficient esterification of Boc-isoleucine anhydride to the polyamide resin with minimum racemisation are *p*-dimethylaminopyridine (0·1 equiv.) and *N*-methylmorpholine (1 equiv.) in dimethylformamide for 30 min. *allo*-Isoleucine formation is less than 2% and the coupling yield 90%. Solvent dimethylformamide gives more rapid acylation than dimethylacetamide. Amino-acid residues coupling faster than

† On sabbatical leave from the University of Ottawa, Canada.

‡ See, for example, peak B in Figure (a) of ref. 3a.

§ The racemisation effected by *p*-dimethylaminopyridine is consistent with the finding (N. L. Benoiton and F. M. F. Chen, unpublished work) that in the presence of triethylamine the symmetrical anhydride of N_α-t-butoxycarbonyl-L-valine gives rise to the 2-t-butoxy-5(4*H*)-oxazolone which racemises when coupled in the presence of the base.[9]

TABLE. Proportion of *allo*- or D-isomer formed in the coupling of urethane-protected amino-acid anhydrides to polydimethylacrylamide resins. Abbreviations: Boc, t-butoxycarbonyl; Fmoc, fluoren-9-ylmethoxycarbonyl; Z, benzyloxycarbonyl; DMA, dimethylacetamide; DMF, dimethylformamide; DMAP, *p*-dimethylaminopyridine.

Amino-acid	Protecting group	Solvent	Equivalents DMAP	Reaction period	% *allo* or D
Ile	Boc	DMA	1	5 h	5
Ile	Boc	DMF	1	5 h	6·5
Ile	Boc	CH_2Cl_2	1	5 h	6
Ile	Fmoc	DMA	1	4 h	4·5
Ile	Z	DMA	1	30 min	5
Ile	Boc	DMA	1[a]	4 h	20
Ile	Boc	DMA	1[b]	30 min	2·5
Ile	Boc	DMF	1[b]	30 min	2·5
Ile	Boc	DMA	1	10 min	2
Ile	Boc	DMA	0·1	5 h	1·5
Leu	Fmoc	DMA	1	3 h	1·5[c]
Phe	Boc	DMA	1	2 h	2[c,d]
Ile	Boc	DMF	0·1[b]	2 × 10 min	2·5
Ile	Boc	DMF	0·1[e]	30 min	1·75

[a] The anhydride was pretreated with DMAP for 5 min, before addition to the resin. [b] The DMAP was added to the resin after the anhydride. [c] D-Amino-acid content determined by subsequent coupling of L- and DL-Boc.Lys(Boc), cleavage, and separation of diastereoisomeric dipeptides.[13] [d] The *p*-carboxybenzyl alcohol resin linkage agent[6c,10] was used and the dipeptide cleaved from the resin by hydrogenolysis. [e] The DMAP and 1 equiv. of *N*-methylmorpholine were added after the anhydride.

isoleucine and valine are expected[12] to give appreciably less racemisation in polar solvents in the absence of special side-chain factors.

Neither the method of Wang[2] (dicyclohexylcarbodi-imide coupling in the presence of *p*-dimethylaminopyridine) nor that of Bodanszky[14] (active ester coupling in the presence of imidazole) were satisfactory when applied to the hydroxymethyl-polydimethylacrylamide support. Low yields were obtained in both cases with racemisation (1·5—2·5% *allo*) in the former. However, it is noteworthy that transesterification of Boc-isoleucine *p*-nitrophenyl ester with hydroxymethyl-polydimethylacrylamide resin in dichloromethane gave no detectable *allo*-isoleucine when catalysed by imidazole or by *p*-dimethylaminopyridine. The yields were 40 and 50% respectively in 24 h. Procedures based on activated esters may, therefore, be particularly appropriate in relatively non-polar polystyrene-based systems which permit use of non-polar solvents, as noted by Bodanszky.[14]

Slight racemisation of the *C*-terminal amino-acid residue is of no consequence when, as with the gastrin peptides cited above, the single resulting diastereoisomer may be separated from the desired optically pure product. Recent experience[15] in the solid-phase synthesis of peptides (carboxy terminus —Thr.Ala.OH) related to histocompatibility antigens[16] has provided another example of a minor contaminant immediately following but separated from the main product by h.p.l.c. which was resistant to enzymic (carboxypeptidase) digestion. On the other hand, in our syntheses of β-endorphin,[3c] analytical evidence was obtained for the formation of a minor byproduct which could not at that time be separated from the immediately preceding major peak. Thus, although racemisation of urethane protected amino-acid derivatives may not be a significant problem in the normal processes of solution or solid-phase peptide synthesis,[8b,9] special circumstances, particularly the use of basic or other catalysts, may require its serious consideration.

Part of this work was financially supported by the Medical Research Council of Canada.

(*Received, 22nd January* 1981; *Com.* 077.)

[1] W. Steglich and G. Höfle, *Angew. Chem., Int. Ed. Engl.*, 1969, **8**, 981.
[2] S. S. Wang and I. D. Kulesha, *J. Org. Chem.*, 1975, **40**, 1227; S. S. Wang, *ibid.*, p. 1235; J. K. Chang, M. Shimizu, and S. S. Wang, *ibid.*, 1976, **41**, 3255.
[3] (a) E. Brown, B. J. Williams, and R. C. Sheppard, *J. Chem. Soc., Chem. Commun.*, 1980, 1093; (b) E. Atherton, V. Woolley, and R. C. Sheppard, *ibid.*, p. 970; (c) E. Atherton, H. Fox, D. Harkiss, and R. C. Sheppard, *ibid.*, 1978, 539.
[4] J. Meienhofer, M. Waki, E. P. Heimer, T. J. Lambros, R. C. Makofske, and C.-D. Chang, *Int. J. Pept. Protein Res.*, 1979, **13**, 35.
[5] J. P. Tam, R. D. DiMarchi, and R. B. Merrifield, *Int. J. Pept. Protein Res.*, 1980, **16**, 412.
[6] (a) E. Atherton, M. J. Gait, R. C. Sheppard, and B. J. Williams, *Bioorg. Chem.*, 1979, **8**, 351; (b) R. Arshady, E. Atherton, D. L. J. Clive, and R. C. Sheppard, *J. Chem. Soc., Perkin Trans.* 1, 1981, 529; (c) E. Atherton, C. J. Logan, and R. C. Sheppard, *ibid.*, p.538.
[7] E. Atherton, H. Fox, D. Harkiss, C. J. Logan, R. C. Sheppard, and B. J. Williams, *J. Chem. Soc., Chem. Commun.*, 1978, 537.
[8] (a) J. H. Jones and M. J. Witty, *J. Chem. Soc., Chem. Commun.*, 1977, 281; (b) J. H. Jones and M. J. Witty, *J. Chem. Soc., Perkin Trans.* 1, 1979, 3203.
[9] (a) N. L. Benoiton and F. M. F. Chen, in 'Peptides: Structure and Biological Function,' eds. E. Gross and J. Meienhofer, Pierce Chemical Co., Rockford, Illinois, 1979, 261; (b) N. L. Benoiton and F. M. F. Chen, *Can. J. Chem.*, 1981, **59**, 384.
[10] R. Arshady, E. Atherton, M. J. Gait, K. Lee, and R. C. Sheppard, *J. Chem. Soc., Chem. Commun.*, 1979, 423.
[11] M. Bodanszky and L. E. Conklin, *Chem. Commun.*, 1967, 773.
[12] (a) N. L. Benoiton and K. Kuroda, *Int. J. Pept. Protein Res.*, 1981, **17**, 197; (b) N. L. Benoiton, K. Kuroda, and F. M. F. Chen, *ibid.*, 1979, **13**, 403.
[13] N. L. Benoiton, K. Kuroda, S. T. Cheung, and F. M. F. Chen, *Can. J. Biochem.*, 1979, **57**, 776.
[14] M. Bodanszky and D. T. Fagan, *Int. J. Pept. Protein Res.*, 1977, **10**, 375.
[15] E. Atherton, W. Hübscher, and V. Woolley, unpublished results.
[16] R. J. Robb, C. Terhorst, and J. Strominger, *J. Biol. Chem.*, 1978, **253**, 5319.