Liquid Phase Peptide Synthesis by Fragment Condensation on Soluble Polymer Support. IV.[1] Relative Reactivities of *N-t*-Butoxycarbonyl-(Boc)amino Acids and Boc-Oligopeptide Acids in Their Esterification with Soluble Chloromethylated Polystyrene

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(Received September 14, 1979)

Synopsis. Relative reactivities of *N-t*-butoxycarbonyl-(Boc)amino acids (Gly, Val, Ile, Leu, Tyr, and Phe) and Boc-oligopeptide acids having various numbers of amino acid residues (n=2, 4, 5, 8, and 10) were determined by competitive esterification in reaction with chloromethylated polystyrene. Increase of peptide-chain lengths reduced reactivity of *C*-terminal carboxylates.

In peptide synthesis on a polymer support, the first step involves an attachment of the *C*-terminal of *N*-protected amino acid to the polymer support *via* a benzyl ester linkage.[2] Studies have been reported on the improvement of this first step in the solid phase peptide synthesis by stepwise elongation.[3-6] However, only a few studies have been made on the esterification step in peptide synthesis by fragment condensation on a polymer support.[6,7] In this paper, we report relative reactivities of Boc-amino acids in the esterification with soluble chloromethylated polystyrene and the influence of peptide-chain lengths of Boc-oligopeptide acids on the reactivity of their *C*-terminal amino acids in the esterification.

Taking advantage of polymer reaction, relative reactivities of Boc-amino acids (Gly, Val, Ile, Leu, Tyr, and Phe)[8] in their esterification with the polymer were determined by competitive esterification with use of amino acid ratios in the acid hydrolysate of the resulting resin (Table 1). We used the recovery of Gly as a standard with the intention of estimating roughly the influence of the *C*-terminal amino acid residue on the esterification. Four methods of esterification were used to obtain information on which differences in reactivity can be compensated: (1) use of potassium carbonate as a base, (2) use of potassium carbonate and 18-crown-6 as a base and catalyst, respectively,[3] (3) use of potassium fluoride as a catalyst,[4] and (4) use of potassium fluoride and 18-crown-6 as catalysts. Reactions proceeded heterogeneously in (1) and (3) and homogeneously in(2) and (4). Relative reactivities of Boc-amino acids in (1) and (3) (heterogeneous reactions) were in line with those in (2) and (4) (homogeneous reactions), respectively. In each case, Boc-amino acids having β-branched substituents (Val and Ile) and a more hydrophobic substituent (Phe) showed higher reactivity.

In order to understand the influence of the peptide-chain lengths of Boc-oligopeptide acids on the reactivity of their *C*-terminal amino acids in esterification, relative reactivities of Boc-oligopeptide acids having various numbers of amino acid residues (n=2, 4, 5, 8, and 10) and the same *C*-terminal amino acid were determined by competitive esterification using Boc-amino acid (Val or Ile) as a reference. The competitive reactions were carried out for the following.

Series A: Boc–Phe–OH (n=1), Boc–Gly–Phe–OH (n=2), Boc–Tyr–Gly_2–Phe–OH (n=4), and Boc–[Tyr(Bzl)–Gly_2–Phe]$_2$–OH (n=8)

Series B: Boc–Leu–OH (n=1), Boc–Leu_2–OH (n=2), Boc–Ala_2–Leu_3–OH[1] (n=5), and Boc–Ala_2–Leu_3–Pro_2–Leu_3–OH[1] (n=10)

Boc-oligopeptide acids of series A and B have Phe and Leu as the *C*-terminal amino acids, respectively.

Methods (1), (2), and (3) were used to estimate the effect of different reactive species on esterification. Relative reactivities of Boc-peptide acids were determined using the ratios of the carboxyl components in the resulting peptide resins taking the recovery of Val or Ile as a standard. The results are shown in Figs. 1 and 2. Total yields in the competitive esterification of the polymer with Boc-peptide acid and Boc-amino acid were 72—95%. In series A, each method showed

TABLE 1. AMINO ACID ANALYSES OF RESINS ESTERIFIED WITH BOC-AMINO ACIDS BY COMPETITIVE REACTIONS AND RELATIVE REACTIVITIES OF BOC-AMINO ACIDS

Esterification method (reaction time, h)	Amino acid content (μmol/g-resin)						Relative reactivities (Gly=1.00)				
	Gly	Val	Ile	Leu	Tyr[a]	Phe	Val	Ile	Leu	Tyr	Phe
K_2CO_3(3)	25	45	45	35	37	44	1.80	1.80	1.40	1.48	1.76
K_2CO_3(70)	72	138	136	103	100	120	1.92	1.89	1.44	1.39	1.67
K_2CO_3+18-crown-6 (1)	46	76	73	62	49	56	1.65	1.59	1.35	1.07	1.22
K_2CO_3+18-crown-6(24)	88	150	142	121	95	114	1.70	1.61	1.37	1.08	1.30
KF (7)	16	23	21	18	20	26	1.44	1.31	1.13	1.25	1.63
KF (70)	72	114	113	80	107	133	1.58	1.60	1.11	1.49	1.85
KF+18-crown-6 (70)	77	109	111	81	110	117	1.42	1.44	1.05	1.42	1.52

a) Tyr decomposes gradually by acid hydrolysis to give low recovery (90—95%) of Tyr. *Cf.* K. Watanabe and K. Inouye, *Bull. Chem. Soc. Jpn.*, **50**, 201 (1977).

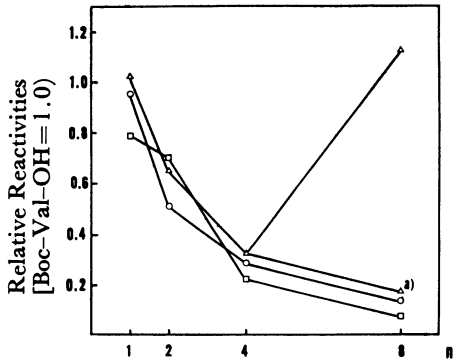

Fig. 1. The influence of the peptide-chain lengths of Boc-oligopeptide acids of series A on the estrification reaction with the chloromethylated polystyrene (Boc-Val-OH was used as a reference for the competitive reactions).
$n=1$, Boc-Phe-OH; $n=2$, Boc-Gly-Phe-OH; $n=4$, Boc-Tyr-Gly_2-Phe-OH; $n=8$, Boc-[Tyr(Bzl)-Gly_2-Phe]$_2$-OH. —○—, K_2CO_3; —□—, K_2CO_3+18-crown-6; —△—, KF; a) KF+18-crown-6.

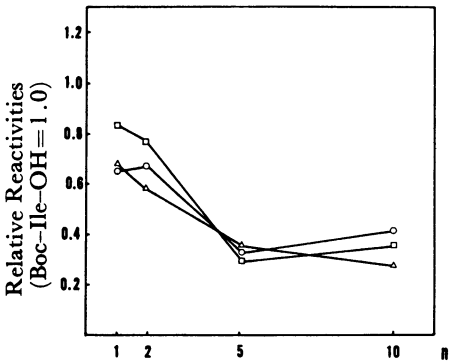

Fig. 2. The influence of the peptide-chain lengths of Boc-oligopeptide acids of series B on the esterification reaction with the chloromethylated polystrene (Boc-Ile-OH was used as a reference for the competitive reactions).
$n=1$, Boc-Leu-OH; $n=2$, Boc-Leu_2-OH; $n=5$, Boc-Ala_2-Leu_3-OH; $n=10$, Boc-Ala_2-Leu_3-Pro_2-Leu_3-OH. —○—, K_2CO_3; —□—, K_2CO_3+18-crown-6; —△—, KF.

the peptide-chain lengths of Boc-peptide acids to decrease the reactivities of their *C*-terminal carboxylates with increase in the number of amino acid residues. In series B, a similar tendency was observed except for Boc-Ala_2-Leu_3-Pro_2-Leu_3-OH, whose secondary structure was estimated to be random coil by the method of Chou and Fasman.[9] That of other Boc-peptide acids ($n=4$, 5, and 8) might be in β-sheet structure.[1] As shown in Fig. 1, an abnormal behavior was observed in the competitive reaction of Boc-Val-OH and Boc-[Tyr(Bzl)-Gly_2-Phe]$_2$-OH with the chloromethylated polystyrene using KF as a catalyst; the amount of Val incorporated was lower (total yield of Boc-Val-OH and Boc-octapeptide, 13.1%) and relative reactivity of Boc-[Tyr(Bzl)-Gly_2-Phe]$_2$-OH higher. Addition of 18-crown-6 to the reaction mixture made the reaction system homogeneous, a similar behavior being observed with methods (1) and (2) (Fig. 1).

Experimental

Relative Reactivities of Boc-amino Acids in Esterification with the Chloromethylated Polystyrene (General Method). Starting with chloromethylated polystyrene (1.00 g; chloromethyl content, 0.89 mmol/g),[10] three-fold equivalents (2.67 mmol) each of Boc-amino acids (Gly, Val, Ile, Leu, Tyr, and Phe) and an equivalent amount of potassium carbonate (8.34 mmol) with the total of the acids were added to 80 ml of DMF. The mixture was allowed to equilibrate at 40 °C in a thermostatted bath (*ca.* 30 min with magnetic stirring), followed by addition of the polymer (1.00 g) and left at 40 °C with stirring. As base and/or catalyst the following compounds were used. Method (2), an equivalent each of potassium carbonate (8.00 mmol) and 18-crown-6 (16.0 mmol). Method (3), an equivalent of anhydrous KF (16.0 mmol). Method (4), an equivalent each of anhydrous KF (16.0 mmol) and 18-crown-6 (16.0 mmol). Portions of the reaction mixture were poured into water at the reaction time given in Table 1. The precipitated resin was filtered off, washed successively with water and methanol in order to remove soluble reactants, and dried *in vacuo* at 50 °C. The resulting resin was subjected to acid hydrolysis with propionic acid–12 M HCl (2 : 1 v/v) for 72 h at 115 °C followed by amino acid analysis.

Relative Reactivities of Boc-oligopeptide Acids in Esterification with the Chloromethylated Polystyrene Using Boc-amino Acid (Val or Ile) as a Reference (General Method). The chloromethylated polystyrene (1.00 g; chloromethyl content, 0.89 mmol/g) was reacted with three equivalents (2.67 mmol) each of Boc-oligopeptide acid and Boc-amino acid in 25 ml of DMF at 40 °C. The reaction times in methods (1), (2), and (3) were 45 h, 24 h, and 45 h, respectively. The work-up was the same as that mentioned above.

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8) Abbreviations for amino acids and protecting groups are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature, *J. Biol. Chem.*, **247**, 977 (1972). Amino acid symbols denote the L-configuration except for Gly. Additional abbreviations: DMF, *N,N*-dimethylformamide.
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