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Liquid Phase Peptide Synthesis by the Fragment Condensation on Soluble Polymer Support. II.[1] The Azide Method

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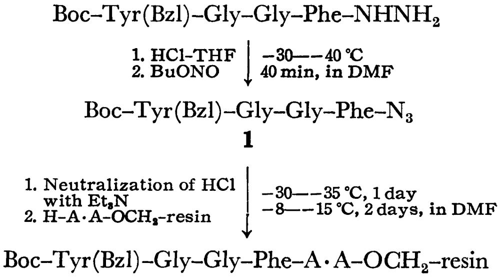
Synopsis. Fragment condensation on a soluble polymer support by the azide method gave rise to the efficient coupling (94—99% yield) of an *N*-protected tetrapeptide azide with the amino acid anchored to the soluble polymer support. Bulkiness of amino acid residues on the polymer support did not affect the reactivity toward the peptide azide.

The azide method[2] is one of the most important coupling procedures for the formation of the peptide bond and has been used for over 70 years. The major reason for its wide use in peptide synthesis is the fact that it is free from racemization during the course of coupling reaction, allowing the synthesis of long peptide fragments with minimum protection on the amino acid side chains.[3] However, the method was not extensively used in solid phase peptide synthesis because the procedure involves a process of isolation or the extraction of the azide, and the coupling yield is not very high (about 50—80%).[4–7] We report here an efficient fragment condensation procedure on the soluble polymer support by a modified azide method of Mazur and Schlatter[8] in a nonaqueous system without the extraction of azide.

In order to test the efficiency of the azide coupling reaction on the soluble polymer support, the tetrapeptide azide, Boc–Tyr(Bzl)–Gly–Gly–Phe–N_3 (**1**),[9] was allowed to react with H–Leu–OCH_2–resin, H–Gly–OCH_2–resin, and H–Val–OCH_2–resin (H–A·A–OCH_2–resin) as shown in Scheme 1.



Scheme 1.

The tetrapeptide hydrazide, Boc–Tyr(Bzl)–Gly–Gly–Phe–$NHNH_2$, was converted into **1** in nonaqueous system (DMF) by treatment with hydrogen chloride followed by butylnitrite at −30—−40 °C for 40 min. After neutralization of hydrogen chloride with triethylamine, H–A·A–OCH_2–resin was reacted with **1** at −30—−35 °C for 1 day and at −8—−15 °C for 2 days. The solution was poured into methanol to precipitate peptide resin. In each case, the recovery of the resulting peptide resin by pouring the reaction mixture into methanol was more than 95% on the assumption that the reaction proceeded quantitatively. Amino acid ratios in acid hydrolysates of the resulting peptide resins are summarized in Table 1. The yields in the coupling reactions of the azide **1** with H–Leu–OCH_2–resin, H–Gly–OCH_2–resin, and H–Val–OCH_2–resin were 95, 99, and 94%, respectively, on the basis of the average values of amino acid ratios.

TABLE 1. AMINO ACID ANALYSES[a] OF Boc–Tyr(Bzl)–Gly–Gly–Phe–A·A–OCH_2–RESINS AND COUPLING YIELDS OF THE AZIDE METHOD

H–A·A–OCH_2–resin	Amino acid content (mmol/g)					Average yield of coupling[b] (%)
	Tyr[c]	Gly	Phe	Leu	Val	
H–Leu–OCH_2–resin	0.37	0.85	0.41	0.44		95
H–Gly–OCH_2–resin	0.31	1.06	0.35			99
H–Val–OCH_2–resin	0.44	0.99	0.49		0.52	94

a) Carried out under the following conditions: resin, 19—23 mg; propionic acid, 4 ml; 12 M HCl, 2 ml; 115 °C; 35 h. b) Determined with use of average values of Gly and Phe contents. c) Acid hydrolysis of tyrosyl peptides usually give low recovery of Tyr.[10]

The results indicate that the azide method enables efficient coupling of the *N*-protected peptide azide with amino free terminals on the soluble polymer support, and that the bulkiness of amino acid residues on amino free terminals does not affect the reactivity of the peptide azide.

Experimental

Soluble chloromethylated polystyrene, H–Leu–OCH_2–resin (Leu content, 0.62 mmol/g), and Boc–Tyr(Bzl)–Gly–Gly–Phe–OMe, prepared previously were used; DMF was purified as reported.[1] Optical rotations were taken in a 1-dm cell on a Jasco Model ORD/UV-5 optical rotatory dispersion recorder. Amino acid analysis was carried out on a Hitachi Liquid Chromatograph, Model 034. Hydrolysis of peptide resins was carried out on 19—23 mg samples of resins with propionic acid–12 M HCl (2:1 v/v) at 115 °C for 35 h.

H-Gly-OCH_2-resin and H-Val-OCH_2-resin. Esterification of the chloromethylated polystyrene with Boc–Gly–OH and Boc–Val–OH was carried out at room temperature for 5 days. After attachment of Gly and Val to the polymer supports, residual benzylic chlorides were removed by the reaction with sodium acetate in DMF at 105 °C for 24 h. The final chlorine contents were less than 0.02 mmol/g. Removal of Boc group with HCl–THF and neutralization of HCl with triethylamine were performed according to the procedure given by Green and Garson.[11] H–Gly–OCH_2–resin: Gly content, 0.41 mmol/g; H–Val–OCH_2–resin: Val content, 0.69

mmol/g (amino acid analysis).

Boc–Tyr(Bzl)–Gly–Gly–Phe–NHNH$_2$. To Boc–Tyr(Bzl)–Gly–Gly–Phe–OMe (2.80 g, 4 mmol) in 30 ml of methanol was added 1 ml of 80% hydrazine hydrate and the solution was allowed to stand for 40 h at room temperature. To this was added 100 ml of ether and the solution was kept in a refrigerator overnight. The precipitate was filtered off to give 1.50 g of a substance, the additional hydrazide (0.58 g) being obtained from the mother liquor. Recrystallization from methanol gave the substance: mp 188—190°C, $[\alpha]^{21}_{D} = +12.4°$ ($c=1.0$, CH_3COOH). Found: C, 62.23; H, 6.31; N, 13.46 %. Calcd for $C_{34}H_{42}N_6O_7$: C, 63.14; H, 6.55; N, 13.00 %. Amino acid ratios: Gly 1.94, Tyr 0.89, and Phe 0.99.

Coupling Reaction of Boc–Tyr(Bzl)–Gly–Gly–Phe–N$_3$ with H–Leu–OCH$_2$–resin, H–Gly–OCH$_2$–resin, and H–Val–OCH$_2$–resin. In a typical experiment, 223 mg (0.36 mmol) of Boc–Tyr(Bzl)–Gly–Gly–Phe–NHNH$_2$ in 20 ml of DMF was cooled to −40 °C with stirring. 565 mg of THF containing 130 mg (3.6 mmol) of hydrogen chloride was added followed by 37 mg (0.36 mmol) of butyl nitrite in 2 ml of DMF. After being kept at −30—−40 °C for 40 min, 400 mg (4.0 mmol) of triethylamine in 3 ml of DMF was added. To the peptide azide solution in threefold excess was then added H–Leu–OCH$_2$–resin (200 mg, Leu content: 0.124 mmol). The mixture was stirred at −30—−40 °C for 30 min and kept at −30—−35 °C for 1 day and −8—−15 °C for 2 days. The reaction mixture was poured into 200 ml of methanol followed by the addition of 30 ml of saturated aqueous sodium chloride in order to precipitate the peptide resin. The precipitates were filtered off, and washed successively with water and methanol in order to remove the soluble reactants. The coupling reactions of Boc–Tyr(Bzl)–Gly–Gly–Phe–N$_3$ with H–Gly–OCH$_2$–resin and H–Val–OCH$_2$–resin were carried out in the same manner. Racemization under the reaction conditions mentioned above was tested by the Izumiya method.[12] Less than 0.5 mol % of Gly–D-Ala–L-Leu was detected by ion exchange chromatography.

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

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