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SOLID-PHASE SYNTHESIS OF PEPTIDES ON THE POLYMERIC SUPPORT TRILAR®

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The solid-phase synthesis of peptides has been achieved using the polymeric support TRILAR® proposed by the Biolar Scientific—Production Association (Olaine).

The improvement of the solid-phase method of synthesizing peptides presupposes the finding of new polymeric supports and suitable anchoring groupings and the possibility of performing the synthesis and splitting off the peptide from the polymer under various conditions.

Recently, polyamide polymeric supports [1], which, unlike cross-linked polymers of the polystyrene type, swell well in both polar and nonpolar solvents, has been used ever more widely in solid-phase peptide synthesis.

In the present work we have studied the applicability for the solid-phase synthesis of peptides of the domestic polymeric support TRILAR® [1]. This consists of a cross-linked polyamide copolymer based on vinylpyrrolidone. The presence of an alkali-labile α-bromopropionyl anchoring group permits N^{α}-protected peptides with a free carboxy group suitable for further synthesis to be obtained under mild conditions.

The α-bromopropionyl group is readily introduced into the amino groups of the polymer by the action of a 1.5-fold excess of the symmetrical anhydride of α-bromopropionic acid at room temperature for 1 h (Table 1). Various analytical tests have shown a high degree of modification of the amino groups in the polymer by bromopropionyl residues. The observed slight excess bromine introduced above the initial amount of amino groups (see Table 1), as shown by the results of elementary analysis for residual bromine (0.05%), does not lead to any complications whatever in the course of further synthesis.

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TABLE 1. Results of the Introduction of α-Bromopropionyl Groups into Batches of Trilar

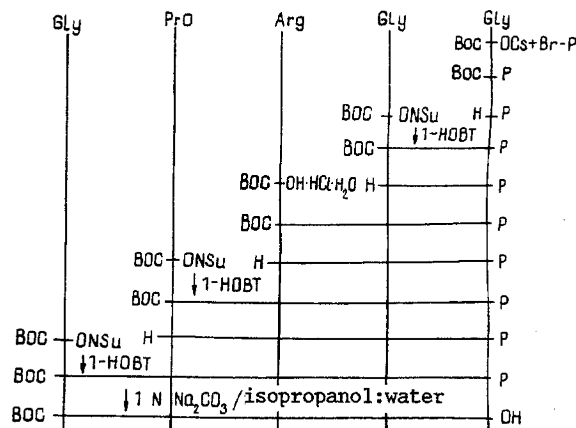
Initial amount of NH_2 groups in the polymer, mmole/g	Residual NH_2 groups (test with picric acid [2]), mmole/g	Semiquantitative ninhydrin test [3]	Amount of Br^- in the polymer	
			% (by elementary analysis)	mmole/g
1,05	0.139	+	8,28	1,035
1,05	Not determined.	+	7,30	0,92
0,76	0,009	+	7,83	0,98
0,66	0,094	+	7,65	0,95

TABLE 2. Conditions and Results of the Synthesis of Boc-Gly-Pro-Arg-Gly-GlyOH on a Copolymer of Styrene with 2% of Divinylbenzene

Activated derivative of Boc-amino acids	Excess of reagents	Reaction time, h	Yield of the condensation reaction, %
BocGlyOCs	3	75	—
BocGlyOPfp	3	24	98
BosArgOH·HCl·H_2O	3	30	98
BocProONSu	3	48	98
BocGlyOPfp	3	24	98

The blocking of the residual amino groups of the polymer after the introduction of the bromopropionyl anchoring groups was done with a fivefold excess of p-nitrophenyl acetate in the presence of an equivalent amount of 1-hydroxybenzotriazole (1-HOBT) [4].

Using the bromopropionylated Trilar, we have synthesized the dipeptide Boc-Val-Phe-OCH_3 (I), which has been described previously [5], and the model peptide Boc-Gly-Pro-Arg-Gly-GlyOH (IIa) (scheme). For comparison, peptide (IIb), corresponding to the same amino acid sequence, was also obtained on a chloromethylated copolymer of styrene with 2% of divinylbenzene* (Table 2).



Synthesis of Boc-Gly—Pro—Arg—Gly—Gly—OH on Trilar.

The C-terminal amino acids were added to the polymers via the corresponding cesium salts [6]. The loads on the supports were 0.38, 0.5, and 0.7 mmole/g, respectively. In all cases, the residual bromopropionyl groups were effectively blocked by the action of a fivefold excess of cesium acetate [7] (see Table 1).

The synthesis of the tripeptide (I) was carried out on Trilar containing 0.98 mmole/g of bromine (see Table 1) by the method of activated pentafluorophenyl esters. As in the

*Ps, from Reanal, Hungary.

synthesis of peptides (II), the splitting out of the N^{α}-Boc group was performed with a 50% solution of TFA in CH_2Cl_2. Because of the alkali-lability of the anchoring group, neutralization was carried out under mild conditions with a 5% solution of Et_3N in DMFA, and the time of treatment was decreased to 5 min. Boc-Val-Phe-OCH_3 was split off from the polymer by transesterification with a 20% solution of Et_3N in methanol [8]. After purification by column chromatography on silica gel, the desired peptide (I) was obtained in high yield (95%), with constants agreeing with those given in the literature [5].

The pentapeptide (IIa) was synthesized on Trilar containing 0.92 mmole/g of bromine (Table 1) by the method of activated N-hydroxysuccinimide esters, with the exception of the introduction of the Arg residue into the peptidylpolymer, for which the derivative Boc-ArgOH·HCl·H_2O with a protonated guanidine function was used. The pentapeptide (IIa) was split off from the polymer under mild conditions by saponification with Na_2CO_3 in aqueous ethanol [8] and, after purification on silica gel, it was obtained with a yield of 30%. According to HPLC, the peptide was a practically individual substance.

The pentapeptide (IIb), synthesized on the chloromethylated copolymer of styrene with 2% of divinylbenzene (Ps) by the activated N-hydroxysuccinimide and pentafluorophenyl ester methods using a scheme similar to that given above (Table 2), was split off from the polymer by saponification with a 0.2 N solution of NaOH in aqueous ethanol, likewise, and, after similar purification, was obtained with a yield of 36%.

Although peptides (IIa) and (IIb) had comparable yields and physicochemical constants, peptide (IIb) was split off from the polymer under more severe alkaline conditions than (IIa). For this reason, in the synthesis of peptides having in their sequences amino acid residues with a higher tendency to undergo racemization, particularly C-terminal ones, mild conditions of splitting out the compounds synthesized from the polymer will play an important role. In view of this, the domestic polymeric support Trilar® proposed by the Biolar Scientific Production Association, which has a number of advantages over the supports commonly used, is promising for use in solid-phase peptide synthesis.

EXPERIMENTAL

In the syntheses we used L-amino acid derivatives from Reanal (Hungary). The individuality of the compounds obtained was confirmed by TLC on Silufol UV-254 (Chemapol) in the systems: 1) chloroform—methanol (9:1); 2) butan-1-ol—pyridine—acetic acid—water (5:5:1:4) and 3) butan-1-ol—acetic acid—water (4:1:5).

On the chromatograms the peptides were revealed with ninhydrin solutions and with the chlorine-tolidine reagent [10]. The completeness of the condensation reactions with the polymer was checked with the aid of a semiquantitative ninhydrin test [3]. The melting points of the substances were determined on a Boëtius heated stage. Angles of specific optical rotation were measured on a Perkin—Elmer 241 mc spectropolarimeter (Sweden).

PMR spectra were obtained in $CDCl_3$ on a Bruker MSL-200 spectrometer (250 MHz). HPLC analyses were conducted on a Gilson-305 chromatograph under the following conditions: column 4.6 × 250 mm support Spherisorb ODS, gradient of 15% → 75% B [sic] in 40 min, A being 0.05 M NaH_2PO_4, pH 3.0, and B 30% of A in acetonitrile; UV detection at 220 nm. IR spectra were taken in paraffin oil on a Shimadzu UR-435 instrument.

The amount of anchoring amino acid in the polymer was determined with the aid of picric acid [2].

Column chromatography was conducted on silica gel (40-100 μm, Chemapol, Czechoslovakia).

The results of the elementary analyses of the compounds synthesized corresponded to the calculated figures.

Methyl Ester of N^{α}-tert-Butoxycarbonylvalylphenylalanyine (I). Trilar containing 0.98 mmole/g of bromine (0.76 mmole/g of initial amino groups) was used. A solution of 1.68 g (4.56 mmole) of Boc-Phe-OCH_3 in 8 ml of DMFA was added to 2 g of the polymer in 8 ml of DMFA, and the mixture was stirred at 20°C for 16 h. The peptidylpolymer was separated off and was washed with DMFA (3 × 15 ml), $CHCl_3$ (3 × 15 ml), MeOH (1 × 15 ml), and $CHCl_3$ (2 × 15 ml). The amount of Boc-Phe-OH in the polymer was 0.38 mmole/g. The residual amino groups in the polymer (0.01 mmole/g) were blocked by treatment with a solution of 0.018 g (0.1 mmole) of p-nitrophenyl acetate and 0.013 g (0.1 mmole) of 1-HOBT in 15 ml of DMFA at 20°C for 12 h. The ninhydrin test was then negative. To replace the residual bromine, the aminoacylpolymer

was stirred with a solution of 1.16 g (6.0 mmole) of ArOCs in 15 ml of DMFA at 40°C for 12 h. Found, %: 0.05.

The deblocking of the α-amino groups in the peptidylpolymer included the following cycle of operations: 1) washing with methylene chloride (2 × 2 min); 2) 50% TFA in CH_2Cl_2 (2 × 20 min); 3) washing with CH_2Cl_2 (2 × 2 min); 4) neutralization with 5% Et_3N in DMFA (2 × 5 min); and 5) washing with DMFA (2 × 2 min). The volume of one wash was 15 ml.

Boc-Val-Phe-P. A solution of 0.87 g (2.28 mmole) of Boc-Val-OPfp in 8 ml of DMFA was added to the N^{α}-deblocked peptidylpolymer, and the reaction mixture was kept at 20°C for 24 h. The peptidylpolymer was separated off in a similar way to Boc-Phe-P. The ninhydrin test was negative.

Boc-Val-Phe-OCH_3. To 0.67 g of Boc-Val-Phe-P was added 6 ml of 20% Et_3N in MeOH, and the mixture was left at 20°C without stirring for 24 h. Then the polymer was separated off and was washed with MeOH (10 ml). The filtrate was evaporated and the residue was purified on a column (1.5 × 40 cm), with elution by chloroform. The yield was 91.6 mg (95.0%); mp 101-102°C $[\alpha]_D^{20}$ +15° (c 1; MeOH); R_f 0.82 (1); IR spectrum, ν_{max}, cm^{-1}: 3600 (NH), 1747 (urethane), 1730 (CO in ester group), 1660 (amide I), 1606 (Ar), 1530 (amide II); PMR (250 MHz, $CDCl_3$): 1.4 (9H, s, Me in tert-Bu), 1.6 (6H, s, Me in iso-Pr), 2.05 (1H, q, N—CH—COO), 3.1 (2H, d, $-CH_2$), 3.7 (3H, s, $-OCH_3$), 3.8-3.9 (1H, t, N—CH—CON), 4.85 (1H, q, —CH in iso-Pr), 5.0; 6.3 (2H, d, 2NH), 7.1-7.3 (5H, m, $-C_6H_5$).

N^{α}-tert-Butoxycarbonylglycylprolylarginylglycylglycine (II). A. A solution of 0.57 g (1.86 mmole) of Boc-Gly-OCs in 5 ml of DMFA was added to 1 g of Trilar containing 0.92 mmole/g of bromine, and the mixture was stirred at 40°C for 4 h and was left at 20°C for 78 h. The aminoacylpolymer was separated off and was washed with DMFA (3 × 5 ml), $CHCl_3$ (1 × 5 ml), MeOH (1 × 5 ml), and $CHCl_3$ (2 × 5 ml). The amount of Boc-Gly-OH in the polymer was 0.5 mmole/g. The replacement of the residual amino groups (0.13 mmole/g) and the residual bromine (0.42 mmole/g) in the polymer was carried out as for compound (I), using 0.12 g (6.55 mmole) of p-nitrophenyl acetate and 0.09 g (6.55 mmole) of 1-hydroxybenzotriazole in 5 ml of DMFA and 1.16 g (6.0 mmole) of AcOCs in 5 ml of DMFA. The ninhydrin test was then negative. Only traces of bromine were found.

Boc-Gly-Gly-P. A solution of 0.46 g (1.68 mmole) of Boc-Gly-ONSu and 0.23 g (1.68 mmole) of 1-HOBT in 5 ml of DMFA was added to 1 g of the N^{α}-deblocked polymer, and the mixture was kept at 20°C without stirring for 24 h. The peptidylpolymer was separated off and was washed in the same way as for compound (I). The ninhydrin test was negative.

Boc-Pro-Arg-Gly-Gly-P. A solution of 0.52 g (1.68 mmole) of Boc-Pro-ONSu and 0.23 g (1.68 mmole) of 1-HOBT in 5 ml of DMFA was added to the N^{α}-deblocked peptidylpolymer, and the mixture was left at 20°C without stirring for 24 h. The peptidylpolymer was separated off and was washed in the same way as for compound (I). The ninhydrin test was negative.

Boc-Gly-Pro-Arg-Gly-Gly-P. A solution of 0.46 g (1.68 mmole) of Boc-Gly-ONSu and 0.23 g (1.68 mmole) of 1-HOBT in 5 ml of DMFA was added to the N^{α}-deblocked polymer, and the mixture was kept at 20°C without stirring for 24 h. The peptidylpolymer was separated off and was washed in the same way as for compound (I). The ninhydrin test was negative.

Boc-Gly-Pro-Arg-Gly-Gly-OH (IIa). A solution of 0.12 g of Na_2CO_3 in 1.1 ml of a 7:3 mixture of isopropanol and water was added to the peptidylpolymer, and the mixture was stirred at 20°C for 3 h. The polymer was separated off and was washed with isopropanol–water (7:3). The filtrate was brought to pH 6 by the addition of AcOH. The solvents were evaporated off, and the residue was purified on a column (3 × 40 cm), with elution of the desired substance by chloroform–methanol (1:9). The yield was 0.10 g (30%, calculated on the initial Gly in the polymer); mp 180-182°C; $[\alpha]_D^{20}$ −64° (c 1; MeOH); R_f 0.48 (2); IR spectrum, ν_{max}, cm^{-1}: 3500 (NH), 3400 (C=NH), 1735 (urethane), 1700 (COOH), 1650 (amide I), 1570 (amide II); quantitative amino acid analysis: Gly 3.3 (3); Arg 1.0 (1); Pro was not determined; HPLC: retention time 13.38 min.

B. Boc-Gly-P. The polymer was chloromethylated polystyrene with 2% of divinylbenzene containing 3.5% of chlorine (Reanal, Hungary). A solution of 2.04 g (6.3 mmole) of Boc-Gly-OCs in 5 ml of DMFA was added to 1 g of the polymer in 5 ml of DMFA, and the mixture was stirred at 40°C for 18 h and was left at 20°C without stirring for 57 h. The aminoacylpolymer was separated off and was washed with DMFA (3 × 7 ml), DMFA–H_2O (1:1) (2 × 7 ml), DMFA (2 × 7 ml), $CHCl_3$ (1 × 7 ml), MeOH (1 × 7 ml), and $CHCl_3$ (1 × 7 ml). The amount of Boc-Gly-

OH in the polymer was 0.7 mmole/g. The blocking of the residual chlorine was achieved in a similar way to the blocking of bromine, starting from 0.7 g (3.75 mmole) of AcOCs in 8 ml of DMFA. Found, %: Cl 2.0. The hydroxymethyl groups were blocked with a solution of 2.24 ml (37.5 mmole) of Ac_2O and 3.0 ml (37.5 mmole) of Et_3N in 3 ml of DMFA for 1 h.

Boc-Gly-Gly-P. A solution of 1.16 g (2.70 mmole) of Boc-Gly-OPfp in 5 ml of DMFA was added to the N^{α}-deblocked peptidylpolymer and the mixture was left at 20°C without stirring for 24 h. Then the peptidylpolymer was separated off and was washed in the same way as for compound (I). The ninhydrin test was negative.

The conditions and results of the synthesis are given in Table 2.

To 0.8 g of the polymer was added 3 ml of dioxane for swelling and then 10 ml of 0.2 N NaOH in 90% EtOH, and the mixture was stirred at 20°C for 3 h. The polymer was separated off and was washed with EtOH. The filtrate was acidified to pH 6. The solvent was evaporated off and the oily residue was purified on a column (2.5 × 40 cm) with elution of the desired substance by methanol—chloroform (1:9). Yield 0.11 g (36.0%); mp 179-180°C; $[\alpha]_D^{20}$ −61°, (c 1; MeOH); R_f 0.14 (3); 0.45 (2); IR spectrum, ν_{max}, cm^{-1}: 3500 (primary NH), 3316 (secondary NH), 3300 (C=NH), 1720 (urethane), 1700 (COOH), 1650 (amide I), 1620 (amide II); quantitative amino acid analysis: Gly 2.6 (3), Arg 1.0 (1); Pro was not determined; HPLC: retention time 13.35 min.

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