THE USE OF THE SILYLATION REACTION IN THE SYNTHESIS OF PEPTIDES FROM L-LYSINE

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In recent years, the silylation reaction has found ever-greater use in organic synthesis [1, 2]. It is beginning to be used ever more widely in the chemistry of amino acids and peptides [2-4]. Amino acids and peptides are readily silylated in aprotic solvents under mild conditions with practically no racemization and with the production of the corresponding trialkylsilyl derivatives with yields close to quantitative. Using these derivatives, a number of peptides have been obtained by the mixed anhydride (MA) and activated ester methods [4].

In the process of silylation, as a rule, the free amino acids or their derivatives pass into solution with the retention of the capacity for forming a peptide bond at the amino group. In our opinion, this property is particularly valuable for the performance of peptide synthesis with amino acids sparingly soluble in organic solvents (such as tyrosine, tryptophan, etc.), and also with some amino acid derivatives (N^{ε}-substituted lysines, N^{ω}- nitroarginines, etc.).

With the aid of the silylation reaction we have synthesized a number of peptides from L-lysine* (Boc-Val-Gly-Lys(Z)-OH; Boc-Val-Lys(Z)-OH; Boc-Lys(Form)-Pro-OH; Boc-Lys(Z)-Lys(Z)-OMe. Such peptides may find use in the synthesis of biologically active substances. The silylation reaction was carried out at room temperature (about 20°C) in methylene chloride or DMFA. The end of conversion was judged from the complete dissolution of the silylated amino acid. As the silylating agent we selected bis(trimethylsilyl)acetamide† (BSA), prepared by a known method [5]. The peptide synthesis was carried out by the MA method using ethyl chloroformate.

The tripeptide Boc-Val-Gly-Lys(Z)-OH was synthesized in two stages via the formation of the intermediate dipeptide Boc-Val-Gly-OH.

The dipeptide Boc-Lys(Form)-Pro-OH was also obtained by the usual method (without the use of the silylation reaction) by coupling Boc-Lys(Form)-OH with H-Pro-OH in water—DMFA with

*All the amino acids used in this work had the L configuration.
†The trimethylsilyl group is denoted subsequently by TMS.

TABLE 1. Physicochemical Characteristics of the Peptides

Peptide synthesized	mp, °C	$[\alpha]_D^{22}$, deg (c 1; MeOH)	Elementary composition, wt.%					
			calculated			found		
			C	H	N	C	H	N
*Boc-Val-Gly-OH	68—70	—26,0	52,55	8,03	10,22	52,38	8,12	9,76
Boc-Val-Gly-Lys(Z)-OH	72—75	—12,0	58,21	7,46	10,45	47,91	7,33	10,30
Boc-Val-Lys(Z)-OH	63—65	—12,0	60,12	7,73	8,77	59,75	7,92	8,10
Boc-Lys(Form)-Pro-OH	Oil	—47,0	—	—	—	—	—	—
†Boc-Lys(Z)-Lys(Z)-OMe	105—108	—12,2	62,20	7,32	8,53	62,00	7,46	8,10

*Also obtained by the following scheme: Boc-Val-OH + H-Gly-Et → Boc-Val-Gly-OEt $\xrightarrow[CH_3OH]{NaOH}$ Boc-Val-Gly-OH; $[\alpha]_D^{22}$ —25° (c 1.0; MeOH).
†Also obtained without the use of the silylation reaction; $[\alpha]_D^{22}$ —11.4° (c 1.0; MeOH).

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high yield (86% of theory); however, the angle of optical rotation was considerably lower ($[\alpha]_D^{22}$ −39.5° (c 1; MeOH)) than that of the product obtained by the silyl method (Table 1). This phenomenon is probably connected with the partial racemization of the lysine residue in the performance of the synthesis by the classical method.

As the work performed has shown, the silylation of AA esters, such as H-Lys(Z)-OH in the production of Boc-Lys(Z)-Lys(Z)-OMe, does not lead to a substantial improvement in peptide synthesis. This dipeptide is obtained by the MA method in approximately the same yield (70-75% of theory) and, judging from the angle of optical rotation, of the same quality with and without the use of silylation.

All the peptides synthesized were obtained in high yield and were characterized by chromatographic homogeneity.

Table 1 gives the physicochemical characteristics of the compounds synthesized.

EXPERIMENTAL

In view of the low hydrolytic stability of the silyl derivatives, the conversions were carried out under conditions excluding the access of moisture. The DMFA was rendered absolute by azeotropic distillation with benzene. The methylene chloride was dried over calcium chloride, distilled, and stored over calcium hydride. The triethylamine was kept over caustic soda for several days and was then boiled over calcium hydride for 8-10 h and distilled. Commercial ethyl chloroformate was subjected to rectification through a column.

The homogeneity of the peptides obtained was established by the TLC method on "Silufol" plates in chloroform–methanol (system 1) and chloroform–acetone–glacial acetic acid (system 2), the ratios being by volume.

1. Preparation of Boc-Val-Gly-OH. In a mixture of 3 g (14.8 mmole) of BSA and 3 ml of CH_2Cl_2, 0.4 g (5.3 mmole) of H-Gly-OH was stirred vigorously in a hermetically closed system at room temperature until the H-Gly-OH had dissolved completely. This gave a solution of TMS-Gly-OTMS.

To a solution prepared from 1.1 g (5 mmole) of Boc-Val-OH and 0.8 ml of Et_3N in 5 ml of CH_2Cl_2, cooled to −15°C, was added 0.6 g (5.5 mmole) of ethyl chloroformate, and the mixture was kept at the same temperature for 15 min.

A solution of TMS-Gly-OTMS was added to the MA so obtained, and the reaction mixture was stirred at −15°C for 1.5 h (monitoring by the TLC method). The solvent was evaporated off in vacuum and the residue was dissolved in ethyl acetate. The solution was washed with 16% citric acid solution and with water, the organic layer was dried, and the solvent was evaporated off in vacuum.

This gave 1.35 g of Boc-Val-Gly-OH, yield 98%, chromatographically homogeneous [R_f 0.20-0.21, system 1 (9:1)]. A similar product was obtained with the same yield by using DMFA as solvent.

2. Preparation of Boc-Val-Gly-Lys(Z)-)H. By the method of paragraph 1, 0.9 g (3.2 mmole) of H-Lys(Z)-OH and 2 g (9.6 mmole) of BSA in CH_2Cl_2 or DMFA give TMS-Lys-OTMS.

A MA was obtained in accordance with paragraph 1 from 0.7 g (2.56 mmole) of Boc-Val-Gly-OH, 0.8 g (2.6 mmole) of ethyl chloroformate, and 0.35 ml of Et_3N. The TMS-Lys-OTMS was mixed with the MA, and the reaction mixture was kept with stirring at from 0 to 5°C for 2 h and at 20°C for 15 h. Then it was treated with a solution of citric acid and with water and, after drying, the solvent was evaporated off in vacuum, the residue was dissolved in a saturated solution of $NaHCO_3$, and the resulting solution was washed with ethyl acetate. Then the aqueous layer was acidified with 2 N HCl at 0-2°C to pH 3-4 and was extracted with CH_2Cl_2. After drying, the solvent was evaporated in vacuum.

This gave 1.1 g of Boc-Val-Gly-Lys(Z)-OH, yield 80%, chromatographically homogeneous [R_f 0.63-0.64, system 2 (7:6:0.15)].

3. Preparation of Boc-Val-Lys(Z)-OH. By the method of paragraph 1, 2 g (7.15 mmole) of H-Lys(Z)-OH and 1.3 g (5.7 mmole) of Boc-Val-OH yielded 2.52 g (92%) of the corresponding dipeptide, chromatographically homogeneous [R_f 0.36-0.39, system (9.5:0.5), twice].

4. Preparation of Boc-Lys(Form)-Pro-OH. By the method of paragraph 1, 5.5 g (20 mmole) of Boc-Lys(Form)-OH and 2.5 g (22 mole) of H-Pro-OH yielded 6.7 g (91%) of the corresponding

dipeptide, chromatographically homogeneous [R_f 0.42-0.43, system 1 (1:1)].

5. Preparation of Boc-Lys(Z)-Lys(Z)-OMe. By the method of paragraph 1, 5.0 g (17 mmol of H-Lys(Z)-OMe and 5.8 g (15.3 mmole) of Boc-Lys(Z)-OH gave, after recrystallization from diethyl ether, 7.5 g (75%) of the corresponding dipeptide, chromatographically homogeneous [R_f 0.40-0.46, system 1 (9:1)].

SUMMARY

1. A number of peptides have been obtained from L-lysine by the application of the silylation method.

2. The peptides obtained can be used as intermediates in the synthesis of biologically active substances.

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INFLUENCE OF ACETYLATION ON THE IODINATION OF THYROGLOBULIN AND THE FORMATION OF THYROXINE

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The synthesis of thyroid hormones in the thyroglobulin molecule includes the condensation of iodotyrosine residues to form iodothyronines [1]. A fundamental role in this process is apparently played by the conformational state of the thyroglobulin [2, 3], since the steri propinquity of the iodotyrosine residues must favor their reaction with one another [1].

Since the charged groups of the protein molecule interact with one another [4], the replacement of such groups by neutral groups should lead to a change in the structure of the protein. The aim of the present work was to determine the influence of such a change on the capacity of thyroglobulin for hormone formation on additional iodination *in vitro*. The replacement of the NH_3^+ groups in thyroglobulin was performed by acetylating the arginine and (or) lysine residues.

2-Diacetylaminocyclohex-2-enone (DACH) is capable of acetylating the bases of α,ω-diamino acids (L-lysine, L-ornithine) with the predominant formation of N^{ω}-acetyl derivatives and conversion into ACH [5]. The capacity for DACH for acetylating the ω-NH_3^+ groups of lysine residues in porcine pepsinogen is also known [6]. In view of this, the acetylation of thryoglobulin was carried out with DACH.

The results of amino acid analyses of the intact and acetylated thyroglobulins are given in Table 1. To calculate the number of residues in the protein we used the molecular weight of 670,000 [1].

Intact thyroglobulin contains 270-280 arginine and 152-168 lysine residues. The acetylated thyroglobulin contained approximately 64 arginine residues less than the original substance. The number of lysine residues scarcely changed. No changes in the amounts of the other amino acids were observed, either.

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