PROTECTION OF THE CARBOXY GROUP IN THE FORM OF THE 2-CYANOETHYL ESTER IN SYNTHESIS OF PEPTIDES

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With the aim of studying the suitability of the 2-cyano group for the protection of carboxylic functions in peptide synthesis, we have obtained the 2-cyanoethyl esters of a number of amino acids and have studied their behavior under the conditions of peptide synthesis. The synthesis of the pentapeptide leucine-enkaphalin has been performed with the use of 2-cyanoethyl protection for C-terminal carboxy groups throughout. The physicochemical characteristics of the compounds synthesized are given.

The 2-cyanoethyl group entered the practices of the synthetic chemistry of the nucleic acids long ago and is widely used for the protection of mono- and diesters of phosphoric acid [1, 2]. This group is stable under the action of acids and is readily removed under mild conditions by dilute alkalies or organic bases in aprotic media through a β-elimination mechanism. In peptide chemistry a whole series of 2-substituted ethyl groups that can be split out by bases through the β-elimination mechnism is also used for the protection of a carboxylic function [3-5], but the 2-cyanoethyl (Cet) group has not been used for this purpose.

In order to study the suitability of the Cet group for the protection of carboxylic functions in peptide synthesis, we have synthesized the Cet esters of a number of tert-butoxycarbonylated (Boc) amino acids and have also effected the synthesis of the peptide leucine-enkaphalin using Cet protection for C-terminal carboxy groups throughout.

The Cet esters of the Boc-amino acids were obtained with high yields by condensing the Boc-amino acids with 3-hydroxypropoinitrile in the presence of dicyclohexylcarbodiimide and catalytic amounts of 4-dimethylaminopyridine and dimethylformamide (DMFA). Characteristics of the Cet esters are given in Table 1. The Cet esters are stable under the action of trifluoroacetic acid (TFAA) and its solutions in chloroform and under the action of triethylamine and N-methylmorpholine in DMFA but are rapidly cleaved by dilute aqueous alkalies (0.1 N NaOH in aqueous DMFA). Particular interest is presented by the possibility of cleaving Cet esters under nonhydrolytic conditions. It is known that the Cet group can be eliminated from a diester of phosphoric acid protected with this group by treating it with 50% triethylamine in pyridine or acetonitrile at room temperature for 30-60 min [2, 6]. It has been found that the Cet esters of Boc-amino acids are considerably more resistant to such conditions — the degree of cleavage does not exceed a few parts per cent in 5-6 h. However, a stronger base — 30% piperidine in DMFA for 10-12 h or 1.2 equivalents of 1,8-diazabicyclo·[5.4.0] undec-7-ene in DMFA for 20-30 min — smoothly splits out the Cet group, giving the corresponding Boc-amino acid in quantitative yields.

TABLE 1. Properties of Some 2-Cyanoethyl Esters of Boc-Amino Acids

Compound	Yield, %	mp, °C	R_f (system 1)	$[\alpha]_D^{27}$, deg (c 2; CH_3OH)
Boc-Gly-OCet	97	62—64	0,63	—
Boc-Leu-OCet	94	43—44	0,80	—34,6
Boc-Phe-OCet	91	56—58	0,71	—7,4
Boc-Ala-OCet	85	68—70	0,66	—33,0*

*c2; DMFA

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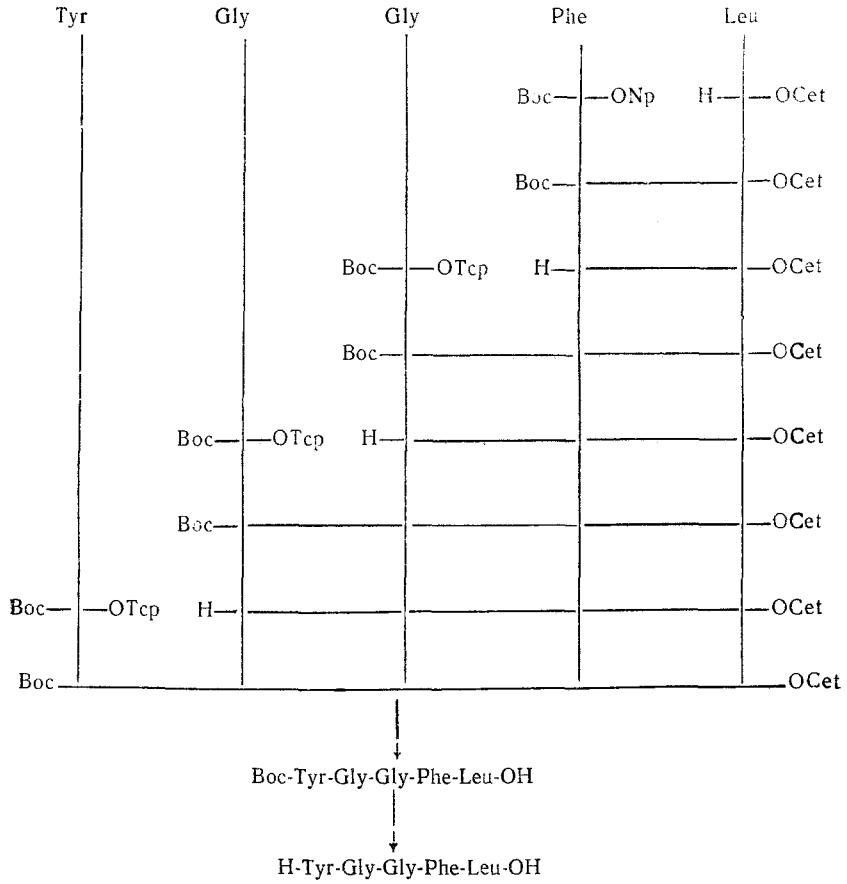

Scheme of the synthesis of leucine-enkephalin

Tcp — 2,4,5-trichlorophenyl; Np — p-nitrophenyl

Leucine-enkephalin was synthesized by the stepwise growth of the peptide chain using activated esters of Boc-amino acids, starting from the Cet ester of Boc-leucine (scheme).

After the elimination of the Boc protection with TFAA, the amino acid or peptide Cet ester trifluoroacetate obtained was acylated with a small excess of an activated Boc-amino acid ester in DMFA in the presence of N-methylmorpholine and 1-hydroxybenzotriazole. After the decomposition of the excess of activated ester with 2-diethylaminoethylamine, the desired peptide was isolated by the usual extraction method. In the last stage, tyrosine was introduced with an unprotected phenolic hydroxyl. The overall yield was 38%, calculated on the initial Cet ester of Boc-leucine. After the removal of the Cet protection by the action of 30% piperidine in DMFA, followed by treatment with TFAA, the protected peptide gave a high yield of leucine-enkephalin. Its structure was confirmed by the results of amino acid analysis and by mass spectra in the regime of accelerated-atom bombardment.

By the use of organic bases in DMFA it is possible to split out the Cet group selectively without affecting other ester groupings in a peptide, such as γ-benzylglutamic acid residues. It is interesting to note that on the hydrazinolysis of Cet esters no β-elimination is observed and the corresponding hydrazides are obtained with good yields. Thus, the hydrazides of Boc-alanine and of Boc-Tyr-Gly-Gly-Phe-Leu were obtained by treating the Cet esters with hydrazine hydrate in methanol.

EXPERIMENTAL

For the synthesis we used L-amino acids, dicyclohexylcarbodiimide, 4-dimethylaminopyridine, 1,8-diazabicyclo[5.4.0]undec-7-ene, and di-tert-butyl pyrocarbonate from Switzerland. 3-Hydroxypropionitrile was redistilled in vacuum. Dimethylformamide was redistilled in vacuum with the addition of ninhydrin and was stored over 4A molecular sieves. Thin-layer chromatography was performed on Kieselgel 60 F_{254} plates in systems 1) benzene—acetone—acetic acid (100:50:2); and 2) chloroform—ethanol—acetic acid (90:20:3). To detect the spots the plates were treated with a ninhydrin or the chlorine /benzidine reagent.

High-performance liquid chromatography was conducted on a LKB instrument using a 4 × 250 mm column with the support LiChrosorb RP-18, 10 μm (Merck), and a linear gradient of from 5 to 90% of acetonitrile in water containing 0.1% of TFAA. The length of the gradient program was30 min, the rate of elution 1 ml/min, and the wavelength of the detector 220 nm. Retetion times t_R were recorded at the output of the integrator. Amino acid analysis was performed on a Biotronik LC 7000 analyzer after the hydrolysis of samples of the peptides in sealed tubes (6 N HCl—TFAA (2: 1), 155°C, 20, 30, and 60 min). Optical rotations were measured on a DIP-360 polarimeter in cells 10 cm long. Mass spectra in the regime of bombardment with accelerated argon atoms were recorded on an MS 7070 HS instrument at an accelerating voltage of 4 kV.

Amino Acid 2-Cyanoethyl Esters. A solution of 5 mmole of a Boc-amino acid and 7.5 mmole of 3-hydroxypropionitrile in 10 ml of DMFA was treated with 0.5 mmole of 4-dimethylaminopyridine and 6 mmole of dicyclohexylcarbodiimide, and the mixture was stirred at 0°C for 2 h. The precipitate of dicyclohexylurea was separated off by filtration and was washed with 50 ml of ethyl acetate. The combined filtrates were washed with saturated NaCl solution, with 5% citric acid, with NaCl solution, with 5% $NaHCO_3$ and again with NaCl solution. The organic phase was dried with anhydrous sodium sulfate and was evaporated; the residue was triturated with hexane and was dried in vacuum. The yields and properties of the esters synthesized are given in Table 1.

Cleavage of the 2-Cyanoethyl Esters. Method 1. After 0.5 mmole of a 2-cyanoethyl ester of an amino acid or peptide had been dissolved in 1 ml of DMFA—piperidine (7:3 by volume), the course of the reaction was monitored by TLC in system 1. The ester was cleaved completely after 10-12 h at room temperature.

Method 2. A solution of 0.5 mmole of the 2-cyanoethyl ester of a Boc-amino acid or peptide in 1 ml of DMFA was treated with 0.6 mmole of 1,8-diazabicyclo [5,4.0] undec-7-ene. Quantitative cleavage of the ester was observed after 20-30 min at room temperature.

Boc-Tyr-Gly-Gly-Phe-Leu-OCet. A solution prepared at 0°C of 1 mmole of Boc-Leu-OCet in 3 ml of TFAA was kept in an ice bath for 10 min. Then it was evaporated in vacuum, and the residue was triturated with dry ether and was dried in vacuum over solid KOH. The resulting H-Leu-OCet trifluoroacetate was dissolved in 2 ml of DMFA, and the solution was treated with 1.2 mmole of Boc-Phe p-nitrophenyl ester and then with 2 mmole of 1-hydroxybenzotriazole and 2 mmole of N-methylmorpholine. After 2 h, 0.5 mmole of 2-dimethylaminoethylamine was added to the mixture and it was kept for another 30 min. Then it was diluted with 30 ml of ethyl acetate, and the organic phase was washed with 5% $NaHCO_3$, with 5% citric acid, and with saturated NaCl solution and it was dried with anhydrous sodium sulfate. The solution was evaporated, and the residue was triturated with ether—hexane (1:1) and was dried in vacuum. The further growth of the peptide chain was carried out similarly in accordance with the scheme shown. The overall yield of Boc-Tyr-Gly-Gly-Phe-Leu-OCet was 38% calculated on the initial Boc-Leu-OCet. mp 112-114°C; R_f 0.40 (system 2); t_R 16.1 min; $[\alpha]_D^{23}$—17.7° (c 2; DMFA). Mass spectrum after dissolution in TFAA: m/z 609 ($M + H^+$).

Tyr-Gly-Gly-Phe-Leu (Leucine-Enkephalin). A solution of 200 mg of Boc-Tyr-Gly-Gly-Phe-Leu-OCet in 2 ml of 30% piperidine in DMFA was kept at room temperature for 12 h. The excess of piperidine was distilled off in vacuum, and the residue was treated with 50 ml of dry ether. The precipitate was filtered off, washed with ether, and reprecipitated from 1.5 ml of acetic acid in 50 ml of ether. The Boc-pentapeptide so obtained (R_f 0.27 (system 2); t_R 14.0 min) was dissolved in 5 ml of TFAA containing 0.3 ml of m-cresol cooled to 0°C, and the solution was kept for 10 min and evaporated. The residue was triturated with ether and was reprecipitated from acetic acid in ether. Yield 139 mg (88%). R_f 0.05 (system 2); t_R 11.1 min (93% of main substance). After chromatography on a column with Sephadex G-15 (25 × 800 mm) in 6% acetic acid, 108 mg of peptide with 98% purity was obtained: $[\alpha]_D^{20}$ + 21.0° (c 1: H_2O). Mass spectrum: m/z 556 ($M + M^+$). Amino acid composition: Tyr 1.00 (1), Gly 1.82 (2), Phe 1.00 (1), Leu 1.00 (1).

Boc-Tyr-Gly-Gly-Phe-Leu-N_2H_3. A solution of 70 mg of Boc-Tyr-Gly-Gly-Phe-Leu-OCet in 1 ml of methanol was treated with 0.025 ml of hydrazine hydrate. After 12 h, 2 ml of ether was added to the mixture and the precipitate was filtered off and was washed with ether. Yield 56 mg (85%). mp 205-207°C (decomp), $[\alpha]_D^{20}$—17.0° (c 1; DMFA), t_R 14.8 min.

SUMMARY

Cet esters of amino acids and peptides selectively cleavable by strong organic bases under nonhydrolytic conditions may be a useful alternative to methyl and benzyl esters in

the preparative synthesis of peptides, particularly in those cases when it is undesirable to use alkaline saponification or catalytic hydrogenation in order to obtain peptides with a free carboxy group. Where necessary, the Cet esters can readily be converted into the hydrazides for the condensation of fragments by the azide method.

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